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(54) Title: NOVEL SURFACE PROTEIN OF NEISSERIA MENINGITIDIS

(57) Abstract

The invention provides a novel surface polypeptide from *Neisseria meningitidis* as well as nucleic acid and nucleic acid sequence homologues encoding this protein. Pharmaceutical compositions containing the polypeptide and nucleic acids of the invention are also disclosed as well as methods useful in the treatment, prevention and diagnosis of *N. meningitidis* infection.

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TITLE

"NOVEL SURFACE ANTIGEN"

FIELD OF THE INVENTION

5 The present invention relates to novel polypeptides as for example obtainable from Neisseria meningitidis, to nucleotide sequences encoding such polypeptides, to the use of these in diagnostics, in therapeutic and prophylactic vaccines and in the design and/or screening of medicaments.

BACKGROUND OF THE INVENTION

Neisseria meningitidis is a Gram-negative bacterium and the causative agent of meningococcal meningitis and septicemia. Its only known host is the human, and it may be carried asymptomatically by approximately 10% of the population (Caugant, D. et al, 1994, Journal of Clinical Microbiology, 32:323-30).

N. meningitidis may express a polysaccharide this allows classification of the capsule, and bacteria according to the nature of the capsule There are at least thirteen serogroups of expressed. N. meningitidis: A,B,C,29-E,H,I,K,L,W135,X,Y and Z, of and C cause which serogroups A, В, 90왕 meningococcal disease (Poolman, J.T. et al, 1995. Infectious Agents and Disease, 4:13-28). Vaccines directed against serogroups A and C are available, but the serogroup B capsular polysaccharide is poorly immunogenic and does not induce protection in humans.

Other membrane and extracellular components are therefore being examined for their suitability for

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inclusion in vaccines. Examples include the outer membrane proteins of classes 1, 2 and 3 (porins), and classes 4 (Rmp) and 5 (Opacity proteins). However, to date, none of these candidates is able to induce complete protection, particularly in children (Romero, J.D., 1994, Clinical Microbiology Review, 7:559-575; Poolman, J.T. et al, 1995, supra).

To create an effective vaccine, necessary to identify components of N. meningitidis which are present in a majority of strains, and which are capable of inducing a protective immune response (bactericidal antibodies). In this regard, reference be made to Brodeur et al. (International may Publication WO 96/29412) who disclose a 22 kDa surface protein which is highly conserved across 99% of all known strains of N. meningitidis. Injection of purified recombinant 22 kDa surface protein protected 80% of immunized mice against development of a lethal infection by N. meningitidis. Notwithstanding the discovery of this protein, there is still a need to isolate more surface proteins of N. meningitidis which are highly conserved across a plurality of strains, and which have immuno-protective profiles against N. meningitidis, and/or which may be used in combination with other components of N. meningitidis to enhance the efficacy of protection against this organism.

SUMMARY OF THE INVENTION

The present inventors have discovered a new gene which is present in all tested strains of N. meningitidis and which encodes a novel polypeptide having a predicted molecular weight of about 62 kDa. Based upon its sequence characteristics and homologies, this polypeptide is predicted to be an

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adhesin and this, together with experimental data suggests that it constitutes a surface protein which may be useful for the production of therapeutic and/or prophylactic vaccines against *N. meningitidis* as described hereinafter.

Accordingly, in one aspect of the invention, there is provided an isolated polypeptide or fragment thereof, or variant or derivative of these, said polypeptide selected from the group consisting of:

10 (a) a polypeptide according to SEO ID NO 2;

- (b) a polypeptide according to SEQ ID NO 5;
- (c) a polypeptide according to SEQ ID NO 7;
- (d) a polypeptide according to SEQ ID NO 9;
- (e) a polypeptide according to SEQ ID NO 11:
- (f) a polypeptide according to SEQ ID NO 13;
- (g) a polypeptide according to SEQ ID NO
 15;
- (h) a polypeptide according to SEQ ID NO 17;
 - (i) a polypeptide according to SEQ ID NO 19; and
 - (j) a polypeptide according to SEQ ID NO 21.

Preferably, said polypeptide, fragment, variant or derivative displays immunological activity against one or more members selected from the group consisting of:-

30 (i) N. meningitidis;

- (ii) said polypeptide;
- (iii) said fragment;
- (iv) said variant; and
- (v) said derivative;

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According to another aspect, the invention provides an isolated nucleic acid sequence encoding a polypeptide or fragment thereof, or variant or derivative of said fragment or polypeptide, according to the first-mentioned aspect. Suitably, said sequence is selected from the group consisting of:

- (1) the nucleotide sequence of SEQ ID NO 1;
- (2) the nucleotide sequence of SEQ ID NO 3;
- (3) the nucleotide sequence of SEQ ID NO 4;
- (4) the nucleotide sequence of SEO ID NO 6;
- (5) the nucleotide sequence of SEQ ID NO 8;
- (6) the nucleotide sequence of SEQ ID NO 10;
- (7) the nucleotide sequence of SEQ ID NO 12;
- (8) the nucleotide sequence of SEQ ID NO 14;
- (9) the nucleotide sequence of SEQ ID NO 16;
- (10) the nucleotide sequence of SEQ ID NO 18;
- (11) the nucleotide sequence of SEQ ID NO 20;
- (12) a nucleotide sequence fragment of any one of SEQ ID NOS 1, 3, 4, 6, 8, 10, 12, 14, 16, 18 and 20; and

(13) a nucleotide sequence homologue of any of the foregoing sequences

Preferably, said sequences encode a product displaying immunological activity against one or more members selected from the group consisting of:-

- (i) N. meningitidis;
- (ii) said polypeptide of the firstmentioned aspect;
- (iii) said fragment of said first-mentioned
 aspect;
- (iv) said variant of said first-mentioned
 aspect; and
- (v) said derivative of said firstmentioned aspect.

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In yet another aspect, the invention resides in an expression vector comprising a nucleic acid sequence according to the second-mentioned aspect wherein said sequence is operably linked to transcriptional and translational regulatory nucleic acid.

In a further aspect, the invention provides a host cell containing an expression vector according to the third-mentioned aspect.

- In yet a further aspect of the invention, there is provided a method of producing a recombinant polypeptide according to the first-mentioned aspect, said method comprising the steps of:
 - (A) culturing a host cell containing an expression vector according to the third-mentioned aspect such that said recombinant polypeptide is expressed from said nucleic acid; and
 - (B) isolating said recombinant polypeptide.
- In a still further aspect, the invention provides an antibody or fragment thereof that binds to one or more members selected from the group consisting of:-
 - (1) N. meningitidis;
 - (2) said polypeptide of the first-mentioned
 aspect;
 - (3) said fragment of the first-mentioned aspect;
 - (4) said variant of the first-mentioned aspect; and
 - (5) said derivative of the first-mentioned aspect.

In yet another aspect, the invention provides a method of detecting N. meningitidis in a biological

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sample suspected of containing same, said method comprising the steps of:-

- (A) isolating the biological sample from a
 patient;
- (B) mixing the above-mentioned antibody or fragment with the biological sample to form a mixture; and
- (C) detecting specifically bound antibody or bound fragment in the mixture which indicates the presence of N. meningitidis.

According to a further aspect, there is provided a method of detecting *N. meningitidis* bacteria in a biological sample suspected of containing said bacteria, said method comprising the steps of:-

- (I) isolating the biological sample from a patient;
- (II) detecting a nucleic acid sequence according to the second-mentioned aspect in said sample which indicates the presence of said bacteria.

The invention further contemplates a method for diagnosing infection of patients by N.

meningitidis, said method comprising the steps of:-

- (1) contacting a biological sample from a patient with a polypeptide, fragment, variant or derivative of the invention; and
- 30 (2) determining the presence or absence of a complex between said polypeptide, fragment, variant or derivative and N. meningitidis-specific antibodies in said sample, wherein the presence of

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said complex is indicative of said infection.

The invention also extends to the use of the polypeptide according to the first-mentioned aspect, the use of the nucleic acids according to the second-mentioned aspect or the use of the antibody or antibody fragment mentioned above in a kit for detecting *N. meningitidis* bacteria in a biological sample.

10 According to a further aspect of the invention, there is provided pharmaceutical a composition comprising isolated polypeptide or an fragment thereof, or a variant or derivative of these, according to the first mentioned aspect.

Preferably, said pharmaceutical composition is a vaccine.

In yet a further aspect, the invention provides a method of preventing infection of a patient by N. meningitidis, comprising the step of administrating a pharmaceutically effective amount of the above-mentioned vaccine.

In a further aspect, the invention provides a method of identifying an immunoreactive fragment of a polypeptide, variant or derivatives according to the first mentioned aspect, comprising the steps of:-

- (a) generating a fragment of said polypeptide, variant or derivative;
- (b) administering said fragment to a
 mammal; and
- 30 (c) detecting an immune response in said which mammal response includes production of elements which meningitidis specifically bind N. and/or said polypeptide, variant

derivative, and/or a protective effect against N. meningitidis infection.

BRIEF DESCRIPTION OF THE DRAWINGS

5 "FIG. 1 depicts plasmid maps and cloning Primers A3A and A3B (SEQ ID NOS 28 and 29, strategy. respectively) were used to amplify from MC58 the region identified in the TIGR database as a homologue of AIDA-I". PCR product was cloned to give pNMAIDA3. Primers A3C (SEQ ID NO 30) and A3D (SEQ ID NO 31) were 10 used in inverse PCR to amplify a 3kbp EagI fragment This product was cloned to give encompassing hiaNm. piEAGA3. piEAGA3 was subcloned to give piEagA3.8 and piEagA3.9. Primers HiaNm:M and HiaNm:P (SEQ ID NOS 22 23, respectively) were used to amplify the 15 contiguous region from MC58 and the product cloned to create pHiaNm. Primers Hia-MBPA (SEQ ID NO 24) and Hia-MBPB (SEQ ID NO 25) were used to amplify the open reading frame of hiaNm, and the product was cloned 20 into pMALC2 to create pMBP-HiaNm;

FIG. 2 is a Southern blot of genomic DNA of a number of strains of N. meningitidis. 2A: serogroup B strains. Lane 1 PMC28, Lane 2 PMC27, Lane 3 PMC25, Lane 4 PMC24, Lane 5 PMC16, Lane 6 PMC13, Lane 7 PMC12, Lane 8 MWt standards, Lane 9 2970, Lane 10 1000, Lane 11 528 Lane 12 SWZ107, Lane 13 H41, Lane 14 H38, Lane 15 NGH36, Lane 16 H15, Lane 17 NGG40, Lane 18 NGF26, Lane 19 NGE30, Lane 20 Lane NGE28 2B: Strains of serogroups other than B. Lane 1 PMC3, Lane 2 PMC17, Lane 3 PMC20, Lane 4 PMC23, Lane 5 PMC8, Lane 6 PMC9, Lane 7 PMC11, Lane 8 PMC14, Lane 9 PMC18, Lane 10 PMC21, Lane 11 PMC29, Lane 12 MWt standards, Lane 13 PMC19, Lane 14 PMC1, Lane 15 PMC6, Lane 16 PMC10, Lane 17 PMC22, Lane 18 PMC26, Lane 19 PMC2. Molecular

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weight markers indicated in kilobase pairs (kb). Genomic DNA was hybridized with a probe corresponding to ntp 276-2054 of SEQ ID NO 1;

FIG. 3 shows a Coomassie stained gel of MBP-HiaNm. Cells containing pMALC2 (Lane 2) or pMBP-HiaNm (Lane 3) after induction with IPTG. Lane 1 molecular weight standards (kDa). Arrows indicate MBP and MBP-HiaNm;

FIG. 4 is a western blot of MC58 and MC58ΔHiaNm proteins incubated with rabbit immune sera. Lane 1; molecular weight standards indicated in kDa, Lane 2 total cellular protein of MC58, Lane 3 total cellular protein of MC58ΔHiaNm Lane 4, OMC preparation of MC58, Lane 5 OMC preparation of MC58ΔHiaNm, each lane contained 50 μL of protein suspension of A₂₈₀= 3.75;

FIG. 5 shows a Coomassie stained gel run in parallel to the gel that was Western blotted in FIG 4. Lanes are the same as for FIG 4;

FIG. 6 shows a sequence comparison of polypeptides of HiaNm, Hia, Hsf using the PILEUF alignment program; and

FIG. 7 shows a sequence comparison of polypeptide sequences of HiaNm from 10 strains of N. meningitidis using the PILEUP program

DETAILED DESCRIPTION OF THE INVENTION

Throughout this specification and the appendant claims, unless the context requires 30 the words "comprise", "comprises" otherwise, "comprising" will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

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Polypeptide sequences

The present invention provides an isolated polypeptide according to SEQ ID NOS 2, 5, 7, 9, 11, 13, 15, 17, 19 and 21, or fragment respectively thereof, or variant or derivative of these. In a preferred embodiment, the polypeptide, fragments, variants and derivatives of the invention display immunological activity against any one member selected from the group consisting of N. meningitidis, said polypeptide, said fragment, said variant and said derivative.

SEQ ID NO 2 corresponds to the novel about 62 kDa surface polypeptide of the hiaNm gene obtained from N. meningitidis strain MC58, as described more fully hereinafter. SEQ ID NOS 5, 7, 9, 11, 13, 15, 17, 19, and 21 correspond to homologous polypeptides deduced from nucleotide sequences obtained from N. meningitidis strains BZ10, BZ198, EG327, EG329, H15, H38, H41, P20, and PMC21, respectively.

For the purposes of this invention, the term "immunological activity" refers to the ability of the aforementioned polypeptide, fragment, variant or derivative to produce an immune response in a mammal to which it is administered, wherein the response includes the production of elements which specifically N.bind meningitidis and/or said polypeptide, fragment, variant or derivative, and/or a protective effect against N. meningitidis infection.

By "isolated" is meant material which is substantially or essentially free from components which normally accompany it in its native state.

By "polypeptide" is meant long chain peptides including proteins.

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As used herein, the term "fragment" includes deletion mutants and small peptides, for example of at least 6, preferably at least 10 and more preferably at least 20 amino acids in length, which comprise antigenic determinants or epitopes. Several such fragments may be joined together. Peptides of this type may be obtained through the application standard recombinant nucleic acid techniques synthesized using conventional liquid or solid phase synthesis techniques. For example, reference may be made to solution synthesis or solid phase synthesis as described, for example, in Chapter 9 entitled "Peptide Synthesis" by Atherton and Shephard which is included in a publication entitled "Synthetic Vaccines" edited by Nicholson and published by Blackwell Scientific Publications. Alternatively, peptides can be produced by digestion of a polypeptide of the invention with proteinases such as endoLys-C, endoArg-C, endoGlu-C staphylococcins V8-protease. The digested fragments can be purified by, for example, high performance liquid chromatographic (HPLC) techniques.

The term "variant" refers to polypeptides in which one or more amino acids have been replaced by different amino acids. It is well understood in the art that some amino acids may be changed to others with broadly similar properties without changing the nature of the activity of the polypeptide (conservative substitutions). Exemplary conservative substitutions in the polypeptide may be made according to the following table:

TABLE 1

Original Residue	Exemplary Substitutions
Ala	Ser

Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro
His	Asn, Gln
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Met	Leu, Ile,
Phe	Met, Leu, Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp, Phe
Val	Ile, Leu

Substantial changes in function are made by selecting substitutions that are less conservative than those shown in TABLE 1. Other replacements would be non-conservative substitutions and relatively fewer of these may be tolerated. Generally, the substitutions which are likely to produce the greatest changes in a polypeptide's properties are those in which (a) a hydrophilic residue (e.g., Ser or Thr) is substituted for, or by, a hydrophobic residue (e.g., Ala, Leu, Ile, Phe or Val); (b) a cysteine or proline is substituted for, or by, any other residue; (c) a residue having an electropositive side chain (e.g., Arg, His or Lys) is substituted for, or by, an electronegative residue (e.g., Glu or Asp) or (d) a residue having a bulky side chain (e.g., Phe or Trp) is substituted for, or by, one having a smaller side chain (e.g., Ala, Ser) or no side chain (e.g., Gly).

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In general, variants will be at least 75% homologous, more suitably at least 80%, preferably at least 85%, and most preferably at least 90% homologous to the basic sequences as for example shown in SEQ ID NOS 2, 5, 7, 9, 11, 13, 15, 17, 19 and 21. 5 is defined as the percentage number of amino acids are identical or constitute conservative substitutions as defined in Table 1. Homology may be determined using sequence comparison programs such as 10 GAP (Deveraux et al. 1984, Nucleic Acids Research 12, 387-395) which is incorporated herein by reference. In this way sequences of a similar or substantially different length to those cited herein may be compared by insertion of gaps into the alignment, such gaps 15 being determined, for example, by the comparison algorithm used by GAP. What constitutes suitable variants may be determined by conventional techniques. example, nucleic acids encoding polypeptides according to SEQ ID NOS 2, 5, 7, 9, 11, 13, 15, 17, 19 20 and 21 can be mutated using either random mutagenesis for example using transposon mutagenesis, or sitedirected mutagenesis. The resultant DNA fragments are then cloned into suitable expression hosts such as E. coli using conventional technology and clones which 25 retain the desired activity are detected. Where the clones have been derived using random mutagenesis techniques, positive clones would have to be sequenced in order to detect the mutation. The term "variant" also includes naturally occurring allelic variants.

By "derivative" is meant a polypeptide which has been derived from the basic sequence by modification, for example by conjugation or complexing with other chemical moieties or by post-translational modification techniques as would be understood in the

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Such derivatives include amino acid deletions art. and/or additions to polypeptides according to SEQ ID NOS 2, 5, 7, 9, 11, 13, 15, 17, 19 and 21 or variants thereof wherein said derivatives retain immunological "Additions" of amino acids may include activity. fusion of the polypeptides or variants thereof with other polypeptides or proteins. In this regard, it will be appreciated that the polypeptides or variants of the invention may be incorporated into larger polypeptides, and such larger polypeptides may also be expected to retain immunological activity against, for example, N.meningitidis. The polypeptides described above may be fused to a further protein, for example, which is not derived from N. meningitidis. The other protein may, by way of example, assist in the purification of the protein. For instance a polyhistidine tag, or a maltose binding protein may be used in this respect as described in more detail Alternatively, it may produce an below. response which is effective against N. meningitidis or it may produce an immune response against another Other possible fusion proteins are those pathogen. which produce an immunomodulatory response. Particular examples of such proteins include Protein A or glutathione S-transferase (GST). In addition, the polypeptide may be fused to an oligosaccharide based vaccine component where it acts as a carrier protein.

Other derivatives contemplated the invention include, but are not limited to, modification to side chains, incorporation unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which constraints conformational on the polypeptides, fragments and variants of the invention.

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Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by acylation with acetic anhydride; acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; amidination with methylacetimidate; carbamoylation of amino groups with cyanate; pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with NaBH₄; reductive alkylation by reaction with aldehyde followed reduction by with NaBH₄; and trinitrobenzylation of amino groups with 2, 4, -6trinitrobenzene sulphonic acid (TNBS).

The carboxyl group may be modified by carbodiimide activation via O-acylisourea formation followed by subsequent derivitization, by way of example, to a corresponding amide.

The guanidine group of arginine residues may be modified by formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

Sulphydryl groups may be modified by methods such as performic acid oxidation to cysteic acid; formation of mercurial derivatives using 4chloromercuriphenylsulphonic acid, 4chloromercuribenzoate; 2-chloromercuri-4-nitrophenol, phenylmercury chloride, other and mercurials; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride other substituted maleimide; carboxymethylation or iodoacetic acid or iodoacetamide; and carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified, for example, by alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphonyl halides or by oxidation with N-bromosuccinimide.

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Tyrosine residues, may be modified by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

The imidazole ring of a histidine residue may be modified by N-carbethoxylation with diethylpyrocarbonate or by alkylation with iodoacetic acid derivatives.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include but are not limited to, use of 4-amino butyric acid, 6-aminohexanoic acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 4-amino-3-hydroxy-6-methylheptanoic acid, t-butylglycine, norleucine, norvaline, phenylglycine, ornithine, sarcosine, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acids contemplated by the present invention is shown in TABLE 2.

TABLE 2

Non-conventional amino acid	Non-conventional amino acid				
α -aminobutyric acid	L-N-methylalanine				
lpha-amino- $lpha$ -methylbutyrate	L-N-methylarginine				
aminocyclopropane-carboxylate	L-N-methylasparagine				
aminoisobutyric acid	L-N-methylaspartic acid				
aminonorbornyl-carboxylate	L-N-methylcysteine				
cyclohexylalanine	L-N-methylglutamine				
cyclopentylalanine	L-N-methylglutamic acid				
L-N-methylisoleucine	L-N-methylhistidine				
D-alanine	L-N-methylleucine				
D-arginine	L-N-methyllysine				
D-aspartic acid	L-N-methylmethionine				
D-cysteine	L-N-methylnorleucine				
D-glutamate	L-N-methylnorvaline				
D-glutamic acid	L-N-methylornithine				
D-histidine	L-N-methylphenylalanine				
D-isoleucine	L-N-methylproline				
D-leucine	L-N-medlylserine				

D-lysine L-N-methylthreonine D-methionine L-N-methyltryptophan D-ornithine L-N-methyltyrosine D-phenylalanine L-N-methylvaline D-proline L-N-methylethylglycine D-serine L-N-methyl-t-butylglycine D-threonine L-norleucine D-tryptophan L-norvaline D-tyrosine α-methyl-aminoisobutyrate D-valine α -methyl- γ -aminobutyrate $D-\alpha$ -methylalanine α-methylcyclohexylalanine $D-\alpha$ -methylarginine α -methylcylcopentylalanine $D-\alpha$ -methylasparagine α -methyl- α -napthylalanine D-α-methylaspartate α-methylpenicillamine N-(4-aminobutyl)glycine D-α-methylcysteine N-(2-aminoethyl)glycine $D-\alpha$ -methylglutamine N-(3-aminopropyl)glycine $D-\alpha$ -methylhistidine D-α-methylisoleucine $N-amino-\alpha-methylbutyrate$ $D-\alpha$ -methylleucine α-napthylalanine N-benzylglycine D-α-methyllysine N-(2-carbamylediyl)glycine $D-\alpha$ -methylmethionine N-(carbamylmethyl)glycine $D-\alpha$ -methylornithiine N-(2-carboxyethyl)glycine $D-\alpha$ -methylphenylalanine N-(carboxymethyl)glycine $D-\alpha$ -methylproline N-cyclobutylglycine $D-\alpha$ -methylserine N-cycloheptylglycine $D-\alpha$ -methylthreonine N-cyclohexylglycine D-α-methyltryptophan N-cyclodecylglycine $D-\alpha$ -methyltyrosine L-a-methylleucine $L-\alpha$ -methyllysine $L-\alpha$ -methylmethionine $L-\alpha$ -methylnorleucine $L-\alpha$ -methylnorvatine L-α-methylornithine $L-\alpha$ -methylproline $L-\alpha$ -methylphenylalanine $L-\alpha$ -methylserine L-a-methylthreonine L-α-methyltryptophan L-α-methyltyrosine L-N-methylhomophenylalanine $L-\alpha$ -methylvaline N-(N-(2,2-diphenylethyl)N-(N-(3,3-diphenylpropyl

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carbamylmethyl)glycine carbamylmethyl)glycine

1-carboxy-1-(2,2-diphenyl-ethyl
amino)cyclopropane

The invention also contemplates covalently modifying a polypeptide, fragment or variant of the invention with dinitrophenol, in order to render it immunogenic in humans

Preferably the invention comprises a polypeptide selected from any one of the polypeptides according to SEQ ID NOS 2, 5, 7, 9, 11, 13, 15, 17, 19 and 21.

- Polypeptides of the inventions may be prepared by any suitable procedure known to those of skill in the art. For example, the polypeptides may be prepared by a procedure including the steps of:
- (a) preparing a recombinant nucleic acid containing a nucleotide sequence encoding a polypeptide according to any one of SEQ ID NOS 2, 5, 7, 9, 11, 13, 15, 17, 19 and 21, or fragment thereof, or variant or derivative of these, which nucleotide sequence is operably linked to transcriptional and translational regulatory nucleic acid;
 - (b) transfecting or transforming a suitable host cell with the recombinant nucleic acid;
 - (c) culturing the host cell to express recombinant polypeptide from said recombinant nucleic acid; and
 - (d) isolating the recombinant polypeptide.

 Suitably said nucleotide sequence is selected from the group consisting of SEQ ID NOS 1, 3, 4, 6, 8, 10, 12, 14, 16, 18 and 20.
- By "recombinant polypeptide" is meant a polypeptide made using recombinant techniques, i.e., through the expression of a recombinant nucleic acid.

The term "recombinant nucleic acid" as used herein refers to nucleic acid formed in vitro by the manipulation of nucleic acid into a form not normally found in nature. In this regard, the recombinant nucleic acid preferably comprises an expression vector which may be either a self-replicating extrachromosomal vector such as a plasmid, or a vector which integrates into a host genome. Generally, such expression vectors include transcriptional translational regulatory nucleic acid operably linked to the said nucleotide sequence.

By "operably linked" is meant that the transcriptional and translational regulatory nucleic acid is positioned relative to the nucleotide sequence encoding the said polypeptide, fragment, variant or derivative in such a manner that such transcription is initiatable. The transcriptional and translational regulatory nucleic acid will generally be appropriate for the host cell used for expression. Numerous types appropriate expression vectors, and suitable of regulatory sequences are known in the art for variety of host cells.

Typically, the transcriptional translational regulatory nucleic acid may include, but is not limited to, promoter sequences, signal sequences, binding ribosomal sites, transcriptional start and sequences, stop translational start and stop sequences, and enhancer or activator sequences.

Constitutive or inducible promoters as known in the art are contemplated by the invention. The promoters may be either naturally occurring promoters, or hybrid promoters which combine elements of more than one promoter.

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In a preferred embodiment, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selection genes are well known in the art and will vary with the host cell used.

The expression vector may also include a fusion partner (typically provided by the expression vector) so that the recombinant polypeptide of the invention is expressed as a fusion polypeptide with said fusion partner. The main advantage of fusion partners is that they assist identification and/or purification of said fusion polypeptide.

In order to express said fusion polypeptide, it is necessary to ligate a nucleotide sequence according to the invention into the expression vector so that the translational reading frames of the fusion partner and the nucleotide sequence of the invention coincide.

Well known examples of fusion partners include, but are not limited to, glutathione-Stransferase (GST), Fc potion of human IgG, maltose binding protein (MBP) and hexahistidine (HIS6), which are particularly useful for isolation of the fusion polypeptide by affinity chromatography. purposes of fusion polypeptide purification affinity chromatography, relevant matrices for affinity chromatography are glutathione-, amylose-, and nickel- or cobalt-conjugated resins respectively. Many such matrices are available in "kit" form, such as the QIAexpress™ system (Qiagen) useful with (HIS₆) fusion partners and the Pharmacia GST purification system.

Another fusion partner well known in the art is green fluorescent protein (GFP). This fusion partner serves as a fluorescent "tag" which allows the

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fusion polypeptide of the invention to be identified by fluorescence microscopy or by flow cytometry. GFP tag is useful when assessing subcellular localization of the fusion polypeptide of invention, or for isolating cells which express the fusion polypeptide of the invention. Flow cytometric methods such as fluorescence activated cell sorting (FACS) particularly useful are in this latter application.

10 Preferably, the fusion partners also have protease cleavage sites, such as for Factor Xa or Thrombin, which allow the relevant protease partially digest the fusion polypeptide the and thereby liberate the invention recombinant polypeptide of the invention therefrom. The liberated 15 polypeptide can then be isolated from the fusion partner by subsequent chromatographic separation.

Fusion partners according to the invention also include within their scope "epitope tags", which are usually short peptide sequences for which a specific antibody is available. Well known examples of epitope tags for which specific monoclonal antibodies are readily available include c-myc, influenza virus haemagglutinin and FLAG tags.

Recombinant polypeptides of the invention may be produced by culturing a host cell transformed with an expression vector containing nucleic acid encoding a polypeptide, fragment, variant or derivative according to the invention. The conditions appropriate for protein expression will vary with the choice of expression vector and the host cell. This is easily ascertained by one skilled in the art through routine experimentation.

Suitable host cells for expression may be prokaryotic or eukaryotic. One preferred host cell

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for expression of a polypeptide according to the invention is a bacterium. The bacterium used may be *Escherichia coli*. Alternatively, the host cell may be an insect cell such as, for example, *SF9* cells which may be utilized with a baculovirus expression system.

The recombinant protein may be conveniently prepared by a person skilled in the art using standard protocols as for example described in Sambrook, et al., MOLECULAR CLONING. A LABORATORY MANUAL (Cold Spring Harbor Press, 1989), incorporated herein by reference, in particular Sections 16 and 17; Ausubel et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (John Wiley & Sons, Inc. 1994-1998), incorporated herein by reference, in particular Chapters 10 and 16; and Coligan et al., CURRENT PROTOCOLS IN PROTEIN SCIENCE (John Wiley & Sons, Inc. 1995-1997) which incorporated by reference herein, in particular Chapters 1, 5 and 6.

20 Nucleotide sequences

The invention further provides a nucleotide which encodes a polypeptide, fragment, variant or derivative as defined above. Suitably said sequence is selected from the group consisting of:-SEQ ID NOS 1, 3, 4, 6, 8, 10, 12, 14, 16, 18 and 20; a nucleotide sequence fragment of any one of SEQ ID NOS 1, 3, 4, 6, 8, 10, 12, 14, 16, 18 and 20; and a nucleotide sequence homologue of the foregoing sequences. Preferably, these sequences encode a product displaying immunological activity as defined above.

As will be more fully described hereinafter, SEQ ID NO 1 corresponds to the *hiaNm* gene obtained from *N. meningitidis* strain MC58. This gene encodes

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the novel 62 kDa (approximately) surface polypeptide of SEQ ID NO 2. SEQ ID NO 3 corresponds to the hiaNm open reading frame sequence of strain MC58, HiaNm. SEQ ID NOS 4, 6, 8, 10, 12, 14, 16, 18, and 20 correspond to the homologous hiaNm open reading frame sequences obtained from N. meningitidis strains BZ10, BZ198, EG327, EG329, H15, H38, H41, P20, and PMC21, respectively.

The term "nucleotide sequence" as used 10 herein designates mRNA, RNA, cRNA, cDNA or DNA.

"nucleotide sequence homologues" The term generally to nucleotide refers sequences which hybridize with а wild-type nucleotide sequence according to the invention under substantially stringent conditions. Suitable hybridization conditions will be discussed hereinafter.

The nucleotide sequence homologues of the invention may be prepared according to the following procedure:

- 20 (i) obtaining a nucleic acid extract from a suitable host;
 - (ii) creating primers which are optionally degenerate wherein each comprises a portion of a wild-type nucleotide sequence of the invention; and
 - (iii) using said primers to amplify, via nucleic acid amplification techniques, one or more amplification products from said nucleic acid extract.

Suitably, the host may be a bacterium. Preferably, the host is from the genus *Neisseria*, more preferably from *N. meningitidis*.

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- (1) 5'-TTAGATTCCACGTCCCAGATT-3' (SEQ ID NO 22);
- (2) 5'-CTTCCCTTCAAACCTTCC-3' (SEQ ID NO 23);
 - (3) 5'-GGTCGCGGATCCATGAACAAATATACCGCAT-3'
 (SEQ ID NO 24);
 - (4) 5'-TCACCCAAGCTTAAGCCCTTACCACTGATAAC-3' (SEQ ID NO 25);
 - (5) 5'-CCAAACCCCGATTTAACC-3' (SEQ ID NO 26);
 - (6) 5'-AATCGCCACCCTTCCCTTC-3' (SEQ ID NO 27);
 - (7) 5'-TTTGCAACGGTTCAGGCA-3' (SEQ ID NO 28);
 - (8) 5'-TATTCAGCAGCGTATCGG-3' (SEQ ID NO
 29);
 - (9) 5'-TGCCTGAACCGTTGCAAA-3' (SEQ ID NO 30); and
 - (10) 5'-CCGATACGCTGCTGAATA-3' (SEQ ID NO 31).

Suitable nucleic acid amplification techniques are well known to the skilled addressee, and include polymerase chain reaction (PCR) as for example described in Ausubel et al. (1994-1998, supra, Chapter 15) which is incorporated herein by reference; strand displacement amplification (SDA) as for example described in U.S. Patent No 5,422,252 which incorporated herein by reference; rolling circle replication (RCR) as for example described in Liu et al., (1996, J. Am. Chem. Soc. 118:1587-1594 and International application WO 92/01813) and Lizardi et al., (International Application WO 97/19193) which are

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herein incorporated by reference; nucleic acid sequence-based amplification (NASBA) as for example described by Sooknanan et al., (1994, Biotechniques **17:**1077-1080) which is incorporated herein reference; and $Q-\beta$ replicase amplification as for example described by Tyagi et al., (1996, Proc. Natl. Acad. Sci. USA 93:5395-5400) which is incorporated herein by reference.

As used herein, an "amplification product"

10 refers to a nucleic acid product generated by nucleic acid amplification techniques.

"Hybridize" or "hybridization" is used here to denote the pairing of complementary bases of distinct nucleotide sequences to produce a DNA-DNA hybrid, a DNA-RNA hybrid, or an RNA-RNA hybrid according to base-pairing rules.

In DNA, complementary bases are:

- (i) A and T; and
- (ii) C and G.
- In RNA, complementary bases are:
 - (i) A and U; and
 - (ii) C and G.

In RNA-DNA hybrids, complementary bases are:

- (i) A and U;
- (ii) A and T; and
 - (iii) G and C.

Typically, substantially complementary nucleotide sequences are identified by blotting techniques that include a step whereby nucleotides are 30 immobilized (preferably a on а matrix synthetic membrane such as nitrocellulose), a hybridization step, and a detection step. Southern blotting is used to identify a complementary DNA sequence; northern blotting is used to identify a complementary RNA

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sequence. Dot blotting and slot blotting can be used to identify complementary DNA/DNA, DNA/RNA or RNA/RNA polynucleotide sequences. Such techniques are well known by those skilled in the art, and have been described in Ausubel et al. (1994-1998, supra) at pages 2.9.1 through 2.9.20.

According to such methods, Southern blotting involves separating DNA molecules according to size by gel electrophoresis, transferring the size-separated DNA to a synthetic membrane, and hybridizing the membrane bound DNA to a complementary nucleotide sequence labeled radioactively, enzymatically or fluorochromatically. In dot blotting and slot blotting, DNA samples are directly applied to a synthetic membrane prior to hybridization as above.

An alternative blotting step is used when identifying complementary nucleotide sequences in a cDNA or genomic DNA library, such as through the process of plaque or colony hybridization. A typical example of this procedure is described in Sambrook et al., (1989, supra) Chapters 8-12.

Typically, the following general procedure can be used to determine hybridization conditions. Nucleotide sequences are blotted/transferred to a synthetic membrane, as described above. A wild type nucleotide sequence of the invention is labeled as described above, and the ability of this labeled nucleotide sequence to hybridize with an immobilized nucleotide sequence analyzed.

A skilled addressee will recognize that a number of factors influence hybridization. The specific activity of radioactively labeled polynucleotide sequence should typically be greater than or equal to about 10⁸ dpm/mg to provide a detectable signal. A radiolabeled nucleotide sequence

of specific activity 10^8 to 10^9 dpm/mg can detect approximately 0.5 pg of DNA. It is well known in the art that sufficient DNA must be immobilized on the membrane to permit detection. It is desirable to have excess immobilized DNA, usually $10\mu g$. Adding an inert polymer such as 10% (w/v) dextran sulfate (MW 500,000) or polyethylene glycol 6000 during hybridization can also increase the sensitivity of hybridization (see Ausubel *supra* at 2.10.10).

10 To achieve meaningful results hybridization between a nucleotide sequence immobilized on a membrane and a labeled nucleotide sequence, a sufficient amount of the labeled nucleotide sequence must be hybridized to the 15 immobilized nucleotide sequence following Washing ensures that the labeled nucleotide sequence hybridized only to the immobilized nucleotide sequences with a desired degree of complementarity to the labeled nucleotide sequence.

20 "Stringency" as used herein, refers to the temperature and ionic strength conditions, and presence or absence of certain organic solvents, during hybridization. The higher the stringency, the higher will be the degree of complementarity between 25 the immobilized nucleotide sequences and the labeled polynucleotide sequence.

"Stringent conditions" designates those conditions under which only nucleotide sequences having a high frequency of complementary bases will hybridize.

Typical stringent conditions include, for example, (1) 0.75 M dibasic sodium phosphate/0.5 M monobasic sodium phosphate/1 mM disodium EDTA/1% sarkosyl at about 42°C for at least 30 minutes; or (2)

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6.0 M urea/0.4 % sodium lauryl sulfate/0.1x SSC at about 42°C for at least 30 minutes; or (3) 0.1x SSC/0.1% SDS at about 68°C for at least 20 minutes; or (4) 1x SSC/0.1% SDS at about 55°C for about 60 minutes; or (5) 1x SSC/0.1% SDS at about 62°C for about 60 minutes; or (6) 1x SSC/0.1% SDS at about 68°C for about 60 minutes; or (7) 0.2X SSC/0.1% SDS at about 55°C for about 60 minutes; or (8) 0.2x SSC/0.1% SDS at about 62°C for about one hour; or (9) 0.2X SSC/0.1% SDS at about 68°C for about 60 minutes. For a detailed example, see CURRENT PROTOCOLS IN MOLECULAR BIOLOGY supra at pages 2.10.1 to 2.10.16, and Sambrook et al. in MOLECULAR CLONING. A LABORATORY MANUAL (Cold Spring Harbour Press, 1989) at sections 1.101 to 1.104, which are hereby incorporated by reference.

While stringent washes are typically carried out at temperatures from about 42°C to 68°C, skilled in the art will appreciate that other temperatures may be suitable for stringent conditions. Maximum hybridization typically occurs at about 20°C to 25° C below the T_m for formation of a DNA-DNA hybrid. It is well known in the art that the T_m is the melting temperature, or temperature at which two complementary polynucleotide sequences dissociate. Methods estimating T_m are well known in the art (see CURRENT PROTOCOLS IN MOLECULAR BIOLOGY supra at page 2.10.8). Maximum hybridization typically occurs at about 10°C to 15°C below the T_m for a DNA-RNA hybrid.

Other stringent conditions are well-known in the art. A skilled addressee will recognize that various factors can be manipulated to optimize the specificity of the hybridization. Optimization of the stringency of the final washes can serve to ensure a high degree of hybridization.

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Methods for detecting labeled nucleotide sequences hybridized to an immobilized nucleotide sequence are well known to practitioners in the art. Such methods include autoradiography, chemiluminescent, fluorescent and colorimetric detection.

Antibodies

The invention also contemplates antibodies against the aforementioned polypeptides, fragments, variants and derivatives. Such antibodies may include any suitable antibodies which bind to or conjugate with a polypeptide, fragment, variant or derivative of invention. For example, the antibodies may Such antibodies may comprise polyclonal antibodies. be prepared for example by injecting a polypeptide, fragment, variant or derivative of the invention into a production species, which may include mice rabbits, to obtain polyclonal antisera. Methods of producing polyclonal antibodies are well known those skilled in the art. Exemplary protocols which may be used are described for example in Coligan et al., CURRENT PROTOCOLS IN IMMUNOLOGY, (John Wiley & Inc, 1991) which is incorporated herein by reference, and Ausubel et al., (1994-1998, supra), in particular Section III of Chapter 11.

In lieu of the polyclonal antisera obtained in the production species, monoclonal antibodies may be produced using the standard method as for example, described in an article by Köhler and Milstein (1975, Nature 256, 495-497) which is herein incorporated by reference, or by more recent modifications thereof as for example, described in Coligan et al., (1991, supra) by immortalizing spleen or other antibody

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producing cells derived from a production species which has been inoculated with one or more of the polypeptides, fragments, variants or derivatives of the invention.

The invention also includes within its scope antibodies which comprise Fc or Fab fragments of the polyclonal or monoclonal antibodies referred to above. Alternatively, the antibodies may comprise single chain Fv antibodies (scFvs) against the peptides of the invention. Such scFvs may be prepared, for example, in accordance with the methods described respectively in United States Patent No 5,091,513, European Patent No 239,400 or the article by Winter and Milstein (1991, Nature, 349 293) which are incorporated herein by reference.

The antibodies of the invention may be used for affinity chromatography in isolating natural or recombinant *N. meningitidis* polypeptides. For example reference may be made to immunoaffinity chromatographic procedures described in Chapter 9.5 of Coligan et al., (1995-1997, supra).

The antibodies can be used to screen expression libraries for variant polypeptides of the invention. The antibodies of the invention can also be used to detect *N. meningitidis* infection described hereinafter.

Detection of N. meningitidis

The presence or absence of *N. meningitidis* in a patient may determined by isolating a biological sample from a patient, mixing an antibody or antibody fragment described above with the biological sample to form a mixture, and detecting specifically bound antibody or bound fragment in the mixture which

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indicates the presence of N. meningitidis in the sample.

The term "biological sample" as used herein refers to a sample which may be extracted, untreated, treated, diluted or concentrated from a patient. Suitably, the biological sample is selected from the group consisting of whole blood, serum, plasma, saliva, urine, sweat, ascitic fluid, peritoneal fluid, synovial fluid, amniotic fluid, cerebrospinal fluid, skin biopsy, and the like.

Any suitable technique for determining formation of the complex may be used. For example, an antibody fragment according antibody or to the invention having a label associated therewith may be Such utilized in immunoassays. immunoassays include, but are not limited to, radioimmunoassays enzyme-linked immunosorbent assays (ELISAs) (RIAs), and immunochromatographic techniques (ICTs) which are well known those of skill in the art. For example, "CURRENT PROTOCOLS reference may be made to IMMUNOLOGY" (1994, supra) which discloses a variety of immunoassays that may be used in accordance with the invention. Immunoassays may competitive assays as understood in the art.

The label associated with the antibody or antibody fragment may include the following:

- i. direct attachment of the label to the antibody or antibody fragment;
- ii. indirect attachment of the label to the antibody or antibody fragment; i.e., attachment of the label to another assay reagent which subsequently binds to the antibody or antibody fragment; and

iii. attachment to a subsequent reaction
 product of the antibody or antibody
 fragment.

The label may be selected from a group including a chromogen, a catalyst, an enzyme, a fluorophore, a chemiluminescent molecule, a lanthanide ion such as Europium (Eu³⁴), a radioisotope and a direct visual label.

In the case of a direct visual label, use may be made of a colloidal metallic or non-metallic particle, a dye particle, an enzyme or a substrate, an organic polymer, a latex particle, a liposome, or other vesicle containing a signal producing substance and the like.

15 A large number of enzymes suitable for use labels is disclosed in United States Patent Specifications U.S. 4,366,241, U.S. 4,843,000, U.S. 4,849,338, all of which are herein incorporated by reference. Suitable enzyme labels useful in the 20 present invention include alkaline phosphatase, horseradish peroxidase, luciferase, β-galactosidase, glucose oxidase, lysozyme, malate dehydrogenase and the like. The enzyme label may be used alone or in combination with a second enzyme which is in solution.

Suitably, the fluorophore is selected from a group including fluorescein isothiocyanate (FITC), tetramethylrhodamine isothiocyanate (TRITL) or R-Phycoerythrin (RPE).

The invention also extends to a method for detecting infection of patients by N. meningitidis, said method comprising the steps of contacting a biological sample from a patient with a polypeptide, fragment, variant or derivative of the invention, and determining the presence or absence of a complex

between said polypeptide, fragment, variant or derivative and *N. meningitidis*-specific antibodies in said serum, wherein the presence of said complex is indicative of said infection.

In a preferred embodiment, detection of the above complex is effected by detectably modifying said polypeptide, fragment, variant or derivative with a suitable label as is well known in the art and using such modified compound in a suitable immunoassay as for example described above.

In another aspect, the invention provides a method of detecting N. meningitidis bacteria in a biological sample suspected of containing bacteria, said method comprising the steps of isolating the biological sample from а patient, detecting a nucleic acid sequence according to the invention in said sample which indicates the presence of said bacteria.

Detection of the said nucleic acid sequence 20 may be determined using any suitable technique. example, a labeled nucleic acid sequence according to the invention may be used as a probe in a Southern blot of a nucleic acid extract obtained from a patient as is well known in the art. Alternatively, a labeled 25 nucleic acid sequence according to the invention may be utilized as a probe in a Northern blot of a RNA extract from the patient. Preferably, a nucleic acid extract from the patient is utilized in concert with oligonucleotide primers corresponding to sense 30 antisense sequences of a nucleic acid sequence according to the invention, or flanking sequences thereof, in a nucleic acid amplification reaction such as PCR, or the ligase chain reaction (LCR) as for described example in International Application 35 W089/09385 which is incorporated by reference herein.

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A variety of automated solid-phase detection techniques are also appropriate. For example, very large scale immobilized primer arrays (VLSIPSTM) are used for the detection of nucleic acids as for example described by Fodor et al., (1991, Science 251:767-777) and Kazal et al., (1996, Nature Medicine 2:753-759). The above generic techniques are well known to persons skilled in the art.

10 Pharmaceutical compositions

A further feature of the invention is the use of the polypeptide, fragment, variant or derivative of the invention ("immunogenic agents") as actives in a pharmaceutical composition for protecting patients against infection by N. meningitidis. Suitably, the pharmaceutical composition comprises a pharmaceutically-acceptable carrier.

By "pharmaceutically-acceptable carrier" is liquid filler, diluent meant a solid or encapsulating substance which may be safely used in systemic administration. Depending upon particular route of administration, a variety pharmaceutically-acceptable carriers, well known in the art may be used. These carriers may be selected from a group including sugars, starches, cellulose and its derivatives, malt, gelatine, talc, calcium vegetable oils, synthetic oils, sulfate, polyols, alginic acid, phosphate buffered solutions, emulsifiers, isotonic saline, and pyrogen-free water.

Any suitable route of administration may be employed for providing a patient with the composition of the invention. For example, oral, rectal, parenteral, sublingual, buccal, intravenous, intraarticular, intra-muscular, intra-dermal, subcutaneous,

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inhalational, intraocular, intraperitoneal, intracerebroventricular, transdermal and the like may be employed. Intra-muscular and subcutaneous injection is appropriate, for example, for administration of immunogenic compositions, vaccines and DNA vaccines.

Dosage forms include tablets, dispersions, suspensions, injections, solutions, syrups, troches, capsules, suppositories, aerosols, transdermal patches and the like. These dosage forms may also include injecting or implanting controlled releasing devices designed specifically for this purpose or other forms implants modified to act additionally in this Controlled release of the therapeutic agent may be effected by coating the same, for example, with hydrophobic polymers including acrylic resins, waxes, higher aliphatic alcohols, polylactic and polyglycolic acids and certain cellulose derivatives hydroxypropylmethyl cellulose. In addition, the controlled release may be effected by using other polymer matrices, liposomes and/or microspheres.

Pharmaceutical compositions of the present invention suitable for oral or parenteral administration may be presented as discrete units such as capsules, sachets or tablets each containing a predetermined amount of one or more therapeutic agents of the invention, as a powder or granules or as solution or a suspension in an aqueous liquid, a nonaqueous liquid, an oil-in-water emulsion or a waterin-oil liquid emulsion. Such compositions may be prepared by any of the methods of pharmacy but all methods include the step of bringing into association one or more immunogenic agents as described above with the carrier which constitutes one or more necessary ingredients. In general, the compositions are

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prepared by uniformly and intimately admixing the immunogenic agents of the invention with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired presentation.

The above compositions may be administered in a manner compatible with the dosage formulation, and in such amount as is immunogenically-effective to protect patients from N. meningitidis infection. dose administered to a patient, in the context of the present invention, should be sufficient to effect a beneficial response in a patient over time such as a reduction in the level of N. meningitidis, or to inhibit infection by N. meningitidis. The quantity of the immunogenic agent(s) to be administered may depend on the subject to be treated inclusive of the age, sex, weight and general health condition thereof. this regard, precise amounts of the immunogenic agent(s) required to be administered will depend on the judgement of the practitioner. In determining the effective amount of the immunogenic agent to be administered in the treatment or prophylaxis against meningitidis, the physician may evaluate circulating plasma levels, progression of disease, and the production of anti-N. meningitidis antibodies. any event, suitable dosages of the immunogenic agents of the invention may be readily determined by those of skill in the art. Such dosages may be in the order of nanograms to milligrams of the immunogenic agents of the invention.

The above compositions may be used as therapeutic or prophylactic vaccines. Accordingly, the invention extends to the production of vaccines containing as actives one or more of the immunogenic

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agents of the invention. Any suitable procedure is contemplated for producing such vaccines. Exemplary procedures include, for example, those described in NEW GENERATION VACCINES (1997, Levine et al., Marcel Dekker, Inc. New York, Basel Hong Kong) which is incorporated herein by reference.

An immunogenic agent according to the invention can be mixed, conjugated or fused with other antigens, including B or T cell epitopes of other antigens. In addition, it can be conjugated to a carrier as described below.

When an haptenic peptide of the invention is used (i.e., a peptide which reacts with cognate antibodies, but cannot itself elicit response), it can be conjugated with an immunogenic carrier. Useful carriers are well known in the art and include for example: thyroglobulin; albumins such as human serum albumin; toxins, toxoids or any mutant crossreactive material (CRM) of the toxin from tetanus, diptheria, pertussis, Pseudomonas, E. coli, Staphylococcus, and Streprococcus; polyamino poly(lysine:glutamic acid); influenza; as Rotavirus VP6, Parvovirus VP1 and VP2; hepatitis B virus core protein; hepatitis B virus recombinant vaccine and the like. Alternatively, a fragment or epitope of a carrier protein or other immnogenic protein may be used. For example, a haptenic peptide of the invention can be coupled to a T cell epitope of a bacterial toxin, toxoid or CRM. In this regard, reference may be made to U.S. Patent No 5,785,973 which is incorporated herein by reference.

In addition, a polypeptide, fragment, variant or derivative of the invention may act as a carrier

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protein in vaccine compositions directed against Neisseria, or against other bacteria or viruses.

The immunogenic agents of the invention may be administered as multivalent subunit vaccines in combination with antigens of N. meningitidis, other organisms inclusive antigens of the pathogenic bacteria H. influenzae, M. catarrhalis, N. gonorrhoeae, E. coli, S. pneumoniae Alternatively or additionally, they may be in concert with oligosaccharide administered or polysaccharide components of N. meningitidis.

The vaccines can also contain a physiologically-acceptable diluent or excipient such as water, phosphate buffered saline and saline.

15 The vaccines and immunogenic compositions may include an adjuvant as is well known in the art. Suitable adjuvants include, but are not limited to: surface active substances such as hexadecylamine, octadecylamine, acid octadecyl amino 20 lysolecithin, dimethyldioctadecylammonium bromide, N, N-dicoctadecyl-N', N'bis (2-hydroxyethylpropanediamine), methoxyhexadecylglycerol, and pluronic polyols; polyamines such as dextransulfate, poly IC carbopol; peptides such as 25 muramyl dipeptide and derivatives, dimethylglycine, tuftsin; oil emulsions; and mineral gels such aluminum phosphate, aluminum hydroxide lymphokines, QuilA and immune stimulating complexes (ISCOMS).

The immunogenic agents of the invention may be expressed by attenuated viral hosts. By "attenuated viral hosts" is meant viral vectors which are either naturally, or have been rendered, substantially avirulent. A virus may be rendered

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substantially avirulent by any suitable physical (e.g., heat treatment) or chemical means (e.g., formaldehyde treatment). By "substantially avirulent" is meant a virus whose infectivity has been destroyed. Ideally, the infectivity of the virus is destroyed without affecting the proteins which carry the immunogenicity of the virus. From the foregoing, it will be appreciated that attenuated viral hosts may comprise live viruses or inactivated viruses.

Attenuated viral hosts which may be useful in a vaccine according to the invention may comprise viral vectors inclusive of adenovirus, cytomegalovirus and preferably pox viruses such as vaccinia (see for example Paoletti and Panicali, U.S. Patent 4,603,112 which is incorporated herein by reference) and attenuated Salmonella strains (see for example Stocker, U.S. Patent No. 4,550,081 which is herein incorporated by reference). Live vaccines particularly advantageous because they lead to prolonged stimulus which can confer substantially long-lasting immunity.

Multivalent vaccines can be prepared from one or more microorganisms that express different epitopes of N. meningitidis (e.g., other surface proteins or epitopes of N. meningitidis). In addition, epitopes of other pathogenic microorganisms can be incorporated into the vaccine.

In a preferred embodiment, this will involve the construction of a recombinant vaccinia virus to express a nucleic acid sequence according to the invention. Upon introduction into a host, the recombinant vaccinia virus expresses the immunogenic agent, and thereby elicits a host CTL response. For example, reference may be made to U.S. Patent No

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4,722,848, incorporated herein by reference, which describes vaccinia vectors and methods useful in immunization protocols.

A wide variety of other vectors useful for therapeutic administration or immunization with the immunogenic agents of the invention will be apparent to those skilled in the art from the present disclosure.

In a further embodiment, the nucleotide sequence may be used as a vaccine in the form of a "naked DNA" vaccine as is known in the art. For example, an expression vector of the invention may be introduced into a mammal, where it causes production of a polypeptide in vivo, against which the host mounts an immune response as for example described in Barry, M. et al., (1995, Nature, 377:632-635) which is hereby incorporated herein by reference.

Detection kits

20 The present invention also provides kits for the detection of N. meningitidis in a biological sample. These will contain one or more particular agents described above depending upon the nature of the test method employed. In this regard, the kits 25 may include one or more of a polypeptide, fragment, variant, derivative, antibody, antibody fragment or nucleic acid according to the invention. The kits may also optionally include appropriate reagents detection of labels, positive and negative controls, 30 washing solutions, dilution buffers and the like. example, a nucleic acid-based detection include (i) a nucleic acid according to the invention (which may be used as a positive control), (ii) an oligonucleotide primer according to the invention, and

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optionally a DNA polymerase, DNA ligase etc depending on the nucleic acid amplification technique employed.

Preparation of immunoreactive fragments

The invention also extends to a method of identifying an immunoreactive fragment of polypeptide, variant or derivatives according to the invention. This method essentially comprises generating a fragment of the polypeptide, variant or derivative, administering the fragment to a mammal; and detecting an immune response in the mammal. response will include production of elements which meningitidis and/or specifically bind N.variant or derivative, polypeptide, and/or а protective effect against N. meningitidis infection.

Prior to testing a particular fragment for immunoreactivity in the above method, a variety of predictive methods may be used to deduce whether a particular fragment can be used to obtain an antibody that cross-reacts with the native antigen. These predictive methods may be based on amino-terminal or carboxy-terminal sequence as for example described in Chapter 11.14 of Ausubel et al., (1994-1998, supra). Alternatively, these predictive methods may be based predictions of hydrophilicity as for described by Kyte and Doolittle (1982, J. Mol. Biol. 157:105-132) and Hopp and Woods (1983, Mol. Immunol. 20:483-489) which are incorporated bv reference herein, or predictions of secondary structure as for example described by Choo and Fasman (1978, Ann. Rev. Biochem. 47:251-276) which is incorporated herein by reference.

Generally, peptide fragments consisting of 10 to 15 residues provide optimal results. Peptides as

small as 6 or as large as 20 residues have worked successfully. Such peptide fragments may then be chemically coupled to a carrier molecule such as keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA) as for example described in Sections 11.14 and 11.15 of Ausubel et al., (1994-1998, supra).

The peptides may be used to immunize an animal as for example discussed above. Antibody titers against the native or parent polypeptide from which the peptide was selected may then be determined by, for example, radioimmunoassay or ELISA as for instance described in Sections 11.16 and 114 of Ausubel et al., (1994-1998, supra).

Antibodies may then be purified from a suitable biological fluid of the animal by ammonium sulfate fractionation or by chromatography as is well known in the art. Exemplary protocols for antibody purification is given in Sections 10.11 and 11.13 of Ausubel et al., (1994-1998, supra).

Immunoreactivity of the antibody against the native or parent polypeptide may be determined by any suitable procedure such as, for example, western blot.

Functional blockers

25 The polypeptides according to SEQ ID NOS 2, 5, 7, 9, 11, 13, 15, 17, 19 and 21 are believed to have adhesin properties. They in fact have some similarity to adhesins of Haemophilus influenzae which are surface antigens. Specifically they are approximately 67% 30 homologous to the Hia protein of H.influenzae (Barenkamp, S. and St. Geme III, J. 1996 Molecular Microbiology 19: 1215-1233), and 74% homologous to the Hsf protein of H. influenzae (St. Geme III, J. et al, 1996, Journal of Bacteriology 178: 6281-6287; and U.S.

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Patent No 5,646,259). For these comparisons, a gap weight of 3, and length weight of 0.01 was used using the GAP program (Deveraux, 1984, supra). sequences of these proteins are illustrated in FIG. 6. interruption of the function of these would be of significant therapeutic polypeptides benefit since they would prevent N. meningitidis bacteria from adhering to and invading Interruption of the function may be effected several ways.

For example, moieties such as chemical reagents or polypeptides which block receptors on the cell surface which interact with a polypeptides according to SEQ ID NOS 2, 5, 7, 9, 11, 13, 15, 17, 19 and 21 may be administered. These compete with the infective organism for receptor sites. Such moieties for example polypeptides comprise of in particular fragments, or functional invention, equivalents of these as well as mimetics.

The term "mimetics" is used herein to refer to chemicals which are designed to resemble particular functional regions of the proteins or peptides. Antiidiotypic antibodies raised against the abovedescribed antibodies which block the binding of the bacteria to a cell surface may also be Alternatively, moieties which interact with receptor binding sites in the polypeptides according to SEQ ID NO 2, 5, 7, 9, 11, 13, 15, 17, 19 and 21 may effectively prevent infection of a cell by N.meningitidis. Such moieties may comprise blocking antibodies, peptides or other chemical reagents.

All such moieties, pharmaceutical compositions in which they are combined with pharmaceutically acceptable carriers and methods of

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treating patients suffering from N. meningitidis infection by administration of such moieties or compositions form a further aspect of the invention.

The polypeptides of the invention may be used in the screening of compounds for their use in the above methods. For example, polypeptides of the invention may be combined with a label and exposed to a cell culture in the presence of a reagent under The ability of reagent to inhibit the binding of the labeled polypeptide to the cell surface can then be observed. In such a screen, the labeled polypeptides may be used directly on an organism such as E. coli. Alternatively, N. meningitidis itself may be engineered to express a modified and detectable form of the polypeptide. The use of engineered N. meningitidis strains in this method is preferred as it is more likely that the tertiary structure of the protein will resemble more closely that expressed in wild-type bacteria.

In order that the invention may be readily understood and put into practical effect, particular preferred embodiments will now be described by way of the following non-limiting examples.

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EXAMPLE 1

Molecular cloning and subcloning and hiaNm mutant construction.

The hiaNm gene was initially isolated by PCR amplification using standard methods. Briefly, due to our previous work on homologues of the AIDA-I protein of E. coli (Jennings, M. et al, 1995, Microbial Pathogenesis, 19: 391-407, Peak, I. et al, Microbial Pathogenesis, in press) we performed a homology

search, identifying a sequence of interest in preliminary data from the project to sequence the genome of MC58¢3 (The Institute for Genomic Research, (ftp://ftp.tigr.org/pub/data/n meningitidis/) 5 amplified the region of homology by PCR (polymerase chain reaction) using oligonucleotides A3A (5'-TTTGCAACGGTTCAGGCA-3', SEQ ID NO 28) and A3B (5' -TATTCAGCAGCGTATCGG-3', SEQ ID NO 29). The resulting 449 base pairs (bp) product was cloned into pT7Blue, to create plasmid pNMAIDA3. 10 To clone the full length gene, further oligonucleotides were designed and used in an inverse PCR reaction. These oligonucleotides were A3C (SEQ ID NO 30) and A3D (SEQ ID NO 31) correspond to the complementary sequence of A3A (SEQ ID NO 28) and A3B (SEQ ID NO 31) respectively. 15 template for this reaction was chromosomal DNA of MC58 which had been restriction digested with EagI and then The resulting 3kbp PCR product was self ligated. cloned into the vector pCRII (Invitrogen), producing plasmid piEagA3. This was digested with EagI 20 EcoRI and the resulting fragments of 1.4kbp and 1.6kbp containing cloned DNA were cloned into pBluescriptSKII, M13minus (Stratagene), resulting in piEagA3.8 and piEagA3.9. Plasmid pHiaNm was generated 25 by PCR amplifying hiaNm and sequence 5' and 3' to it using oligonucleotide (5' primers HiaNm:P TTAGATTCCACGTCCCAGATT-3', SEQ ID NO 22) and HiaNm:M (5'-CTTCCCTTCAAACCTTCC-3', SEO ID NO 23), corresponding to nucleotide position (ntp) 113-133 and 2102-2085 respectively of SEQ ID NO 1, and cloning the 30 product into pT7Blue. Plasmid pHiaNm∆Kan was created by insertion of a kanamycin resistance cassette into the unique BalII site of pHiaNm corresponding to ntp 680 of SEQ ID No 1. The kanamycin resistance cassette

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excised from pUC4Kan (Pharmacia) with BamHI. pHiaNm Δ Kan was transformed into N. meningitidis strain MC58 by incubating bacteria with plasmid DNA for 3 hours on Brain Heart Infusion agar Manufacturer's Inc) supplemented with 10% heated horse blood ("BHI plates") at 37°C in 5% CO₂. A single colony was picked onto fresh selective media, grown, and used for further studies. This mutant strain is designated MC58 Δ HiaNm. Disruption of the hiaNm gene in this strain was confirmed by Southern blot using a probe corresponding to ntp 276-2054 of SEQ ID NO 1.

EXAMPLE 2

Nucleotide sequence analysis

15 Nucleotide sequence analysis was performed using the PRISM Dye terminator sequencing Kit with AmpliTaq DNA polymerase FS or BigDye terminator sequencing kit as suggested by the manufacturer's instructions (Perkin Elmer), in conjunction with a 20 model 373a automated sequencer (Applied Biosystems). For each strain, hiaNm was amplified in three independent PCR reactions using primers HiaNm5'A2: 5'-CCAAACCCCGATTTAACC-3' (SEQ ID NO 26) and HiaNm3'A: 5'-AATCGCCACCCTTCCCTTC-3' (SEQ ID NO 27), as indicated on 25 FIG. 1, and corresponding to ntp 230-247 and 2114-2097 of SEQ ID No 1, and the resulting products purified This was used as template for direct and pooled. sequencing on both strands. Data were analysed using the GCG programs (Deveraux et al. (1984) Nucleic Acids Research 12, 387-395) and AssemblyLIGN (Oxford 30 Molecular). Several oligonucleotides were generated as necessary to complete sequences. Sequences of hiaNm of 10 strains are shown in SEQ ID NOS 1, 3, 4,

6, 8, 10, 12, 14, 16, 18, and 20, and the deduced amino acid sequences of those genes are shown in SEQ ID NO 2, 5, 7, 9, 11, 13, 15, 17, 19 and 21.

Comparison of hiaNm from these strains indicated that they share 90-99% identity with hiaNm 5 of MC58. In addition, hiaNm of MC58 is 62% and 68% homologous to hia and hsf of Haemophilus influenzae However, in the strains examined, hiaNm is 1770-1800 This is markedly different from the hia and 10 hsf which are 3294 and 7059 bp long respectively. predicted polypeptide of hiaNm, HiaNm, also exhibits homology to several other bacterial proteins, including AIDA-I, the adhesin involved in diffuse adherence of the diarrhoeagenic Escherichia strain 2787 (0126:H27), HMW1, another Haemophilus 15 adhesin, UspAl, a high molecular weight protein of Moraxella catarrthalis, and SepA involved in tissue flexneri invasion of Shigella (Benz, I. Schmidt, M.A., 1992, Molecular Microbiology 6:1539-20 1546, Barenkamp, S.J. and Leininger, E. 1992, Infection Immunity 60: 1302-1313, Aebi,C. 1997, Infection and Immunity 65: 4367-4377, Benjelloun-Touimi, Z et al 1995, Molecular Microbiology 17:123-135). Homology to these (and several other proteins) 25 occurs over the first fifty amino acids of HiaNm. Analysis of this sequence reveals the presence of a predicted signal sequence, with cleavage sites at amino acid 50 in all HiaNm sequences examined. long signal sequences are common to proteins located 30 in the outer membrane of Gram-negative bacteria (Henderson, I et al, 1998, Trends in Microbiology 6: 370-8). The proteins mentioned above to which the first fifty amino acids of HiaNm is homologous are all members of the "autotransporter" outer-membrane

protein family (Henderson, I, supra). This strongly suggests that HiaNm is located in the outer membrane of N. meningitidis.

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EXAMPLE 3

Southern blot analysis

Southern blot analysis was performed using standard techniques (Sambrook et al., supra, Ausubel et al., supra). Briefly, genomic DNA was prepared 10 from 70 strains of N. meningitidis of several serogroups, restriction digested ar.d separated electrophoretically on an agarose gel prior to capillary transfer to a nylon membrane. membranes were hybridized with a labeled probe. 15 probe used corresponded to ntp 276-2054 of SEQ ID NO 1, encompassing the entire open reading frame of hiaNm strain MC58. This was labeled with (dioxygenin) according to manufacturer's instructions (Boehringer Mannheim). Stringent washes 20 performed (two washes of 5 minutes at 22°C in 2 x SSC/0.1% SDS followed by two washes of 30 minutes, 68°C, 0.2 x SSC/0.1% SDS). Hybridization was detected colorimetrically using nitro-blue-tetrazolium/ bromochloryl-indolyl-phosphate (NBT/BCIP) as recommended by 25 the manufacturer. Signals were detected in all strains examined. (FIG. 2 for example). to the prototypic strain MC58, the following strains were investigated:-

30 TABLE 3

Strain Name			Strain name	Source	
PMC 3 (J1079)	2 [*]	A	NGF26	1	В

PMC17 (K874)	2	A	NGG40	1	В
PMC 20 ((H79)	2	A	н15	1	В
PMC23 (K750)	2	A	SWZ107	1	В
PMC 12 (K852)	2	В	528	1	В
PMC 13 (K859)	2	В	2970	1	В
PMC 16 (K873)	2	В	1000	1	В
PMC 24 (K782)	2	В	MPJB28	3 ^c	В
PMC 25 (K791)	2	В	MPJB56	3	В
PMC 27 (K816)	2	В	MPJB88	3	В
PMC 28 (K837)	2	В	MPJB157	3	В
BZ10	1 ^B	В	MPJB328	3	В
BZ47	1	В	MPJB627	3	В
BZ83	1	В	MPJB820	3	В
BZ133	1	P	MPJB945	3	В
BZ147	1	В	PMC 8 (K157)	2	С
BZ163	1	В	PMC 9 (K497)	2	С
BZ169	1	В	PMC 11 (K848)	2	С
BZ198	1	В	PMC 14 (K860)	2	С
BZ232	1	В	PMC 18 (K879)	2	C
NG3/88	1	В	PMC 21 (K656)	2	С
NG4/88	1	В	PMC 29 (K841)	2	С
NG6/88	1	В	MPJC05	3	С
EG327	1	В	MPJC14	3	С
EG329	1	В	MPJC154	3	С
DK353	1	В	мрјс302	3	С
179/82	1	В	мрјс379	3	c
66/84	1	В	PMC19	2	W
DK24	1	В	MPJW025	3	w
NGH36	1	В	PMC 1 (J603)	2 .	x
н38	1	В	PMC 6 (K131)	2	х
H41	1	В	PMC 10 (K526)	2	Y
NGE28	1	В	PMC 22 (K685)	2	Y
NGE30	1	В	PMC 26 (K810)	2	Y
NGP20	1	В	PMC 2 ((J1049)	2	z

A World Health Organization Collaborating Centre for Reference and Research on Meningococci, Oslo, Norway

B Public Health Laboratory Service Meningococcal

⁵ Reference Laboratory, Manchester, UK

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^c Brisbane Hospitals, now in strain collection of M.P. Jennings, Department of Microbiology, University of Queensland, Brisbane, Australia.

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EXAMPLE 4

Expression and partial purification of MBP-HiaNm

plasmid vector was constructed Α permitted the expression of a protein consisting of a 10 fusion of Maltose Binding Protein and HiaNm (MBP-HiaNm). The plasmid pHiaMBP was generated by amplifying hiaNm from MC58 using primers Hianm-MBPA 5'-GGTCGCGGATCCATGAACAAAATATACCGCAT-3' (SEQ ID NO 24) and HiaNm-MBPB 5'-TCACCCAAGCTTAAGCCCTTACCACTGATAAC-3' 15 (SEQ ID NO 25). These primers encompass the start and stop codons of hiaNm of N. meningitidis strain MC58 and engineered restriction sites for ease of cloning. Plasmid restriction maps and positions oligonucleotides are shown in FIG. 1. The resultant 20 PCR product was ligated into BamHI/HindIII restriction digested plasmid pMALC2 (New England Biolabs), and the resultant plasmid, pHiaMBP (See FIG. 1) reintroduced E. coli strain DH5 α . This E. coli strain containing pHiaMBP was induced to express the HiaNm-25 MBP fusion protein under conditions recommended by the manufacturer (New England Biolabs). Cell extracts from cultures containing pHiAMBP were separated by 10% SDS-PAGE, and the fusion protein was purified by elution using the Mini-Gel Electro-eluter 30 according to manufacturer's instructions. Fractions containing the HiaNm-MBP fusion protein were detected by Western blot using rabbit anti-MBP sera (New England Biolabs). The purity of the HiaNm-MBP

fusion protein was determined by SDS-PAGE followed by Coomassie staining, and the amount of recovered protein estimated by BCA assay (Sigma) or absorbance at a wavelength of 280nm.

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EXAMPLE 5

Generation of polyclonal sera

partially purified HiaNm-MBP fusion The protein obtained in Example 4 was used to generate polyclonal sera in rabbits. Samples of eluted HiaNmMBP fusion protein were dialyzed against sterile phosphate buffered saline pH 7.4, (PBS) (Sigma). This was then mixed with adjuvant (MPL+TDM+CWS, Sigma), concentration of 50-150µg/mL and inoculated at two weekly intervals into two New Zealand White rabbits. Blood from these rabbits. Serum was taken extracted by clotting at room temperature for one hour 4°C before followed by overnight incubation at centrifugation at 4000 x rpm at 4°C. The supernatant was removed and re-centrifuged. Serum was stored in aliquots at -80°C. Sera obtained were used in bactericidal assays and Western blots (see below).

To test the specificity of the sera obtained, analysis was undertaken. Western blot Briefly, proteins of N. meningitidis strains MC58 MC58ΔHianm were separated electrophoretically on SDS-PAGE before electrophoretic transfer to nitrocellulose membrane using a Semi-Dry Blotter (BioRad). incubated sequentially with then and alkaline-phosphatase conjugated anti-Rabbit IgG (Sigma) before colorimetric detection with NBT/BCIP (Sigma). These experiments demonstrated that antibodies were elicited by the HiaNm-MBP fusion protein which

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were specific for, and detected a band in, MC58 but in MC58 Δ HiaNm (see FIG. 4). The predicted molecular weight of the deduced polypeptide of HiaNm is 62.3 kDa. The band detected by the sera migrates at an apparent MW in excess of 150 kDa. At least three of the homologous "autotransporter" proteins reported in the literature also display such anomalous migration: the high molecular weight outer membrane proteins UspA1 and UspA2 of Moraxella catarrhalis have predicted molecular weights of 62.5 kDa and 88.3 kDa respectively but migrate at 85 kDa and 120 kDa, respectively and as the UspA complex at between 350 kDa and 720 kDa (Aebi, C. et al., 1997, Infection and Immunity, 65: 4367-4377, Klingman, K.L. and Murphy, T.F., 1994, Infection and Immunity, 62: 1150-1155). Similarly, Hia of Haemophilus influenzae has predicted molecular weight of 116 kDa but when expressed in a phage, Hia migrates at greater than 200 kDa (Barenkamp, S. and St. Geme III, J. 1996 Molecular Microbiology 19: 1215-1233).

In order to confirm that HiaNm is associated with the outer membrane of N. meningitidis, outer membrane complexes (omc) were prepared, essentially as previously described (van der Ley, P. et al, 1991, 25 Infection and Immunity, **59:**2963-71). Briefly, bacteria were grown overnight on Brain Heart Infusion agar (Acumedia Manufacturer's Inc) supplemented with 10% heated horse blood BHI plates, resuspended in 10 mM Tris pH 8.0 and heat killed, before sonication to 30 disrupt the membrane. Cellular debris were removed by centrifugation at 10,000 Х (rcf, relative q centrifugal force), and the supernatant recentrifuged at 50,000 x g. This pellet was resuspended in 1% sarkosyl/10 mM Tris pH8.4 and centrifuged at 10,000 x

The supernatant was centrifuged at $75,000 \times g$ and g. the pellet resuspended in Tris pH 8.4, quantification spectrophotometrically at a wavelength 280nm. An aliquot of the sarkosyl-insoluble 5 fraction, which contains outer membrane proteins, $(50\mu l \text{ of } A_{280}=3.75)$ was subjected to SDS-PAGE Western blotted as described above. The results, shown in FIG. 4 demonstrate that reactivity with the anti-HiaNmMBP antisera is observed with wild type MC58, but 10 with MC58∆HiaNm, in not which hiaNm has inactivated. The increase in reactivity with the anti-HiaMBP sera observed between whole cell samples, and the omc samples containing the same amount of total protein, in MC58 cultures is consistent with the membrane association of HiaNm. 15

EXAMPLE 6

Bactericidal assay

To determine whether the anti-HiaMBP antisera 20 contained bactericidal antibodies specific for HiaNm, bactericidal assays were performed with wild type MC58 and MC58∆HiaNm. This assay was performed by a modification of the method described by Hoogerhout et. al. (1995, Infection and Immunity, 63: 3473-3478). 25 Briefly, MC58 and MC58∆HiaNm were grown overnight on BHI plates at 37°C in 5% CO2. Bacteria from this overnight culture were subcultured under the same conditions for 4-6 hours before suspension in 1 mL PBS. Numbers of bacteria were estimated by lysis of a 30 sample in 0.2N NaOH/1% SDS and absorbance at a wavelength of 260 nm, where $A_{260}=1 = 10^9$ cfu/mL. bacterial suspension was adjusted to approximately 105 cfu/mL in PBS. Rabbit sera to be tested was heat

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inactivated at 56°C for 45 minutes. Serum from four week old, New Zealand White rabbits was pooled and used as a source of complement (Central Animal Breeding House, University of Queensland). The assav was carried out in sterile polystyrene flat-bottomed 96 well microtitre plate. The total volume in each well was 24 μ L: 12 μ L of twofold serially diluted serum in PBS and 6 μL of bacterial suspension (containing between 300-900 bacteria). Sera and bacteria were incubated at room temperature for 10 minutes before addition of 6 µL of 80% complement in PBS (final concentration 20% vol/vol). Controls were a) PBS, bacteria and complement, b) PBS, bacteria and serum. After addition of all components and mixing, a 7 µL aliquot from each control well was spread on a BHI plate. The microtitre plate was then incubated at 37°C in 5% CO2 for 60 minutes. After this incubation, a 7 μL aliquot from each well was spread on BHI plates. All BHI plates were then incubated for 14-18 hours at 37° C in 5% CO₂, and bacterial colonies counted. bactericidal killing is reported as the highest reciprocal dilution at which at least 90% of bacteria Serum used was from the same rabbit and were killed. same test bleed as used for Western blot experiments as reported in Example 5 above. experiments consistently demonstrated reduced titers (approximately 3 fold, Table 4) of killing against MC58ΔHiaNm in comparison to the wild type strain, MC58, indicating that the anti-HiaMBP antisera contained bactericidal antibodies specific for HiaNm.

TABLE 4

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	The second secon	

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MC58	12 (+/- 4.6)
MC58ΔHiaNm	3.5 (+/- 1)

a Mean of four independent experiments

DISCUSSION

Repetitive DNA has been associated with 5 virulence determinants in some pathogenic bacteria. Southern blots using such a repetitive DNA motif revealed the presence of at least three loci which contained this motif in N. meningitidis strain MC58 10 (Peak, I. et al., 1996, FEMS Microbiology Letters, **137:**109-114). These genes were cloned and sequence analysis of two of these repeat associated loci (nmrep2 and nmrep3) revealed open reading frames of approximately 670 amino acids (Jennings, M. et al, 15 1995, Microbial Pathogenesis, 19: 391-407, Peak, I. et al, Microbial Pathogenesis, in press). exhibited homology to each other and homology to the carboxyl-terminal of the adhesin AIDA-I of E. coli. AIDA-I is 1286 amino acids long. The carboxyl-20 terminal region constitutes a putative outer membrane transport domain and the amino-terminal domain of the mature protein constitutes the adhesin domain. amino-terminal domain crosses the membrane through the putative transport domain and is designated the 25 passenger domain.

As Nmep2 and Nmep3 share sequence homology with the transporter domain of AIDA-I, they are thought to form membrane pores. Nmrep2 and Nmrep3 are approximately half the size of AIDA-I, and are homologous to the membrane spanning domain of AIDA-I. We hypothesized that there existed in N. meningitidis

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a locus with homology to the amino-terminal domain of AIDA-I. We searched for such a homologue in the data from the project to sequence N. meningitidis strain MC58¢3 (TIGR, supra) and found one region with homology to a gene designated AIDA-I in Haemophilus influenzae strain Rd (HI1732) because of its homology to AIDA-I of E. coli, (Fleischmann et. al., 1995 Science 269:496-512,). In view of the homologies noted above, the applicants decided to investigate further.

The gene was initially isolated by PCR amplification of the DNA corresponding to the 471 base pair fragment, named gnmaa84r, from N. meningitidis MC58 3 and the sequence was confirmed. Further PCR experiments enabled larger fragments to be amplified. These were cloned and sequence analysis undertaken as shown in FIG 1. The gene exhibited homology to the amino-terminal region of AIDA-I of E. coli and we designated it aida3, as it represented the third AIDA-I homologue in N. meningitidis (with nmrep2 and nmrep3). Since then, the discovery of two further genes, hia and hsf from H. influenzae has been published (Barenkamp, S. and St. Geme III, J. 1996 Molecular Microbiology 19: 1215-1233, St. Geme III, J. et al, 1996, Journal of Bacteriology 178: 6281-6287), to which aida3 is more similar. We have therefore redesignated this gene hiaNm. (HI1732, the H. influenzae gene first identified as an homologue of AIDA-I has also been re-designated hia in light of the reports of Barenkamp and St. Geme III).

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Throughout the specification the aim has been to describe the preferred embodiments of the invention without limiting the invention to any one embodiment or specific collection of features. It will therefore

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be appreciated by those of skill in the art that, in light of the instant disclosure, various modifications and changes can be made in the particular embodiments exemplified without departing from the scope of the present invention. All such modifications and changes are intended to be included within the scope of the appendant claims

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CLAIMS

- 1. An isolated polypeptide or fragment thereof, or variant or derivative of these, said polypeptide selected from the group consisting of:
- (a) a polypeptide according to SEQ ID NO 2;
 - (b) a polypeptide according to SEQ ID NO 5;
 - (c) a polypeptide according to SEQ ID NO 7;
 - (d) a polypeptide according to SEQ ID NO 9;
 - (e) a polypeptide according to SEQ ID NO 11;
 - (f) a polypeptide according to SEQ ID NO 13;
 - (g) a polypeptide according to SEQ ID NO 15;
 - (h) a polypeptide according to SEQ ID NO 17;
 - (i) a polypeptide according to SEQ ID NO 19; and
- 15 (j) a polypeptide according to SEQ ID NO 21.
 - 2. A polypeptide, fragment, variant or derivative according to claim 1, displaying immunological activity against one or more members selected from the group consisting of:-
 - (i) N. meningitidis;
 - (ii) said polypeptide;
 - (iii) said fragment;
 - (iv) said variant; and
- 25 (v) said derivative;
 - 3. A polypeptide, fragment, variant or derivative according to claim 1, displaying immunological activity against N. meningitidis.
 - 4. An isolated nucleic acid sequence encoding a polypeptide or fragment thereof, or variant or derivative of these, said polypeptide selected from the group consisting of:

Substitute Sheet (Rule 26) RO/AU

	(a) a polypeptide according to SEQ ID NO 2;
	(b) a polypeptide according to SEQ ID NO 5;
	(c) a polypeptide according to SEQ ID NO 7;
	(d) a polypeptide according to SEQ ID NO 9;
5	(e) a polypeptide according to SEQ ID NO 11;
	(f) a polypeptide according to SEQ ID NO 13;
	(g) a polypeptide according to SEQ ID NO 15;
	(h) a polypeptide according to SEQ ID NO 17;
	(i) a polypeptide according to SEQ ID NO 19;
10	and
	(j) a polypeptide according to SEQ ID NO 21.
	5. An isolated nucleic acid sequence according
	to claim 4, encoding a product displaying
15	immunological activity against one or more members
	selected from the group consisting of:-
	(i) N. meningitidis;
	<pre>(ii) said polypeptide;</pre>
	<pre>(iii) said fragment;</pre>
20	(iv) said variant; and
	<pre>(v) said derivative.</pre>
	6. An isolated nucleic acid sequence according
	to claim 4, encoding a product displaying
25	immunological activity against N. meningitidis.
	7. An isolated nucleic acid sequence selected
	from the group consisting of:
	(1) the nucleotide sequence of SEQ ID NO 1;
30	(2) the nucleotide sequence of SEQ ID NO 3;
	(3) the nucleotide sequence of SEQ ID NO 4;
	(4) the nucleotide sequence of SEQ ID NO 6;
	(5) the nucleotide sequence of SEQ ID NO 8;
	(6) the nucleotide sequence of SEQ ID NO 10;
.35	(7) the nucleotide sequence of SEQ ID NO 12;

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- (8) the nucleotide sequence of SEQ ID NO 14;
- (9) the nucleotide sequence of SEQ ID NO 16;
- (10) the nucleotide sequence of SEQ ID NO 18;
- (11) the nucleotide sequence of SEQ ID NO 20;
- (12) a nucleotide sequence fragment of any one of SEQ ID NOS 1, 3, 4, 6, 8, 10, 12, 14, 16, 18 and 20; and
 - (13) a nucleotide sequence homologue of any of the foregoing sequences

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- 8. A nucleic acid sequence according to claim 7, encoding a product displaying immunological activity against one or more members selected from the group consisting of:-
- 15 (i) N. meningitidis;
 - (ii) said polypeptide;
 - (iii) said fragment;
 - (iv) said variant; and
 - (v) said derivative.

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- 9. A nucleic acid sequence according to claim 7, encoding a product displaying immunological activity against N. meningitidis.
- 25 10. The nucleic acid sequence of claim 7, wherein said homologue is obtained from the genus Neisseria.
 - 11. The nucleic acid sequence of claim 5 or claim
 - 7, wherein said homologue is obtained from a strain of
- 30 N. meningitidis.
 - 12. A method of obtaining a nucleotide sequence homologue comprising the steps of:-
 - (i) obtaining a nucleic acid extract from a suitable host;

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- (ii) creating primers which are optionally degenerate wherein each comprises a portion of a nucleic acid sequence according to claim 5 or claim 7; and (iii) using said primers to amplify, via a nucleic acid amplification technique, one or more amplification products from said nucleic acid extract.
- 10 13. The method of claim 12, wherein said nucleic acid extract is obtained from the genus *Neisseria*.

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- 14. The method of claim 12, wherein said nucleic acid extract is obtained from a strain of N.
 15 meningitidis.
 - 15. The method of claim 12, wherein said primers are selected from the group consisting of SEQ ID NOS 22, 23, 24, 25, 26, 27, 28, 29, 30, and 31.
- 16. The method of claim 12, wherein the nucleic acid amplification technique is PCR.
- 17. An expression vector comprising a nucleic acid sequence according to claim 4 or claim 7, wherein said sequence is operably linked to transcriptional and translational regulatory nucleic acid.
- 18. A host cell transfected or transformed with an expression vector comprising a nucleic acid sequence according to claim 4 or claim 7, wherein said sequence is operably linked to transcriptional and translational regulatory nucleic acid.

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19.	A	method	of	producing	a	recombinant
polypeptic	le	comprising	the	steps of:		

- (A) culturing a host cell according to claim
 18 such that said recombinant
 polypeptide is expressed from said
 nucleic acid; and
- (B) isolating said recombinant polypeptide.
- 20. An antibody or antibody fragment which binds to one or more members selected from the group consisting of:-
 - (1) N. meningitidis;

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- (2) a polypeptide according to claim 1;
- (3) a fragment of said polypeptide;
- (4) a variant of said polypeptide or said fragment; and
- (5) a derivative of said polypeptide or said fragment.
- 20 21. The antibody of claim 20, wherein said antibody or antibody fragment binds N. meningitidis.
 - 22. A method of detecting *N. meningitidis* in a biological sample suspected of containing same, said method comprising the steps of:-
 - (A) isolating the biological sample from a
 patient;
 - (B) mixing the antibody or antibody fragment of claim 20 or claim 21 with the biological sample to form a mixture; and
 - (C) detecting specifically bound antibody or bound fragment in the mixture which indicates the presence of N. meningitidis.

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23. detecting Α method of N.meningitidis bacteria in a biological sample suspected of containing said bacteria, said method comprising the steps of:-

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- (I) isolating the biological sample from
 a patient;
- (II) detecting a nucleic acid sequence according to claim 4 or claim 7 in said sample which indicates the presence of said bacteria.
- 24. A method for diagnosing infection of patients by N. meningitidis, said method comprising the steps of:-
 - (1) contacting a biological sample from a patient with a polypeptide, fragment, variant or derivative according to claim 1; and
 - (2) determining the presence or absence of a complex between said polypeptide, fragment, variant or derivative and N. meningitidis-specific antibodies in said sample, wherein the presence of said complex is indicative of said infection.
- 25. Use of the polypeptide, fragment, variant or derivative according to claim 1 for the manufacture of a kit for the detection or diagnosis of N. meningitidis infection in humans.
 - 26. Use of the nucleic acid sequence according to claim 4 or claim 7 for the manufacture of a kit for

the detection or diagnosis of *N. meningitidis* infection in humans.

- 27. Use of one or more oligonucleotide primers selected from the group consisting of SEQ ID NOS 22, 23, 24, 25, 26, 27, 28, 29, 30 and 31, and optionally a thermostable polymerase, in a kit for the detection or diagnosis of *N. meningitidis* infection in humans.
- 10 28. Use of the antibody or antibody fragment according to claim 20 or claim 21 for the manufacture of a kit for the detection or diagnosis of N.

 meningitidis infection in humans.
- 15 29. Use of a pharmaceutically effective amount of a polypeptide, fragment, variant or derivative according to claim 1 for the prevention or treatment of N. meningitidis infection in humans.
- 30. Use of a pharmaceutically effective amount of an antibody or antibody fragment according to claim 20 or claim 21 for the prevention or treatment of N. meningitidis infection in humans.
- 25 31. A pharmaceutical composition comprising an isolated polypeptide or fragment thereof, or a variant or derivative of these, according to claim 1.
- 32. The pharmaceutical of claim 31, which is a vaccine.
 - 33. A method of preventing or treating infection of a patient by N. meningitidis, comprising the step

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of administrating a pharmaceutically effective amount of a vaccine according to claim 32.

34. A method of identifying an immunoreactive fragment of a polypeptide, variant or derivatives according to claim 1, comprising the steps of:-

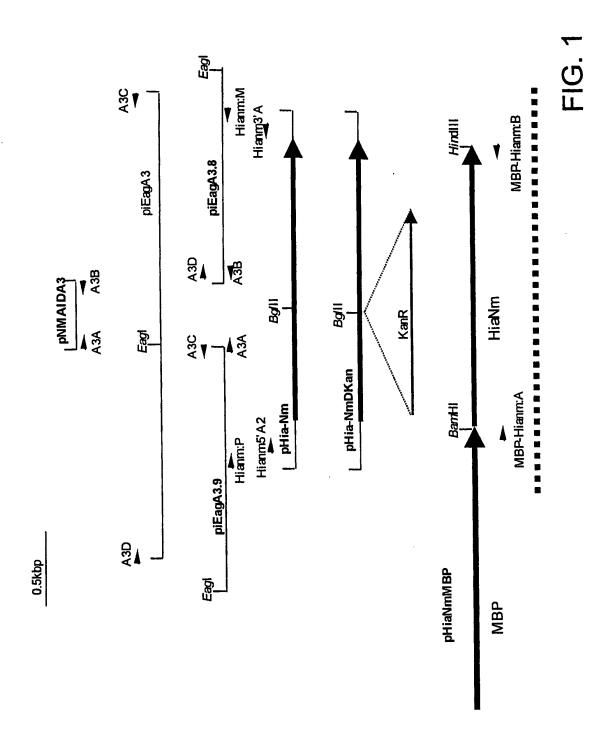
10

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- (a) generating a fragment of said polypeptide, variant or derivative;
- (b) administering said fragment to a mammal; and

detecting an immune response in said mammal which response includes production of elements which specifically bind *N. meningitidis* and/or said polypeptide, variant or derivative, and/or a protective effect against *N. meningitidis* infection.

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1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

FIG. 2A

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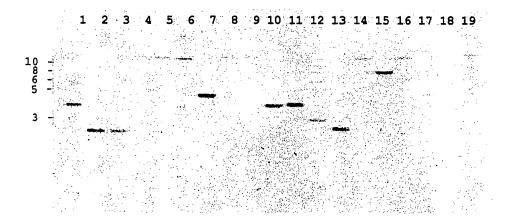


FIG. 2B

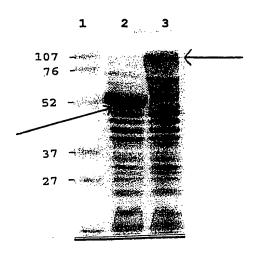


FIG. 3

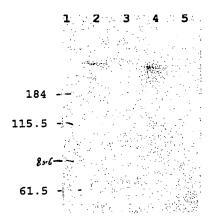


FIG. 4

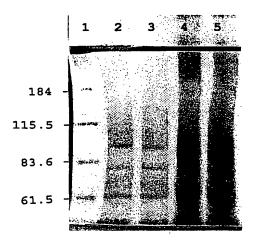


FIG. 5

FIG. 6 Hsf MNKIFNVIWN VMTQTWVVVS ELTRTHTKRA SATVETAVLA TLLFATVQAN Hia MNKIFNVIWN VVTQTWVVVS ELTRTHTKCA SATVAVAVLA TLLSATVEAN HiaNm MNKIYRIIWN SALNAWVVVS ELTRNHTKRA SATVKTAVLA TLLFATVQAS Hsf ATDEDEELDP VVRTAPVLSF HSDKEGTGEK EVTENSNWGI YFDNKGVLKA Hia HiaNm A..... Hsf GAITLKAGDN LKIKONTDES TNASSFTYSL KKDLTDLTSV ATEKLSFGAN Hsf GDKVDITSDA NGLKLAKTGN GNVHLNGLDS TLPDAVTNTG VLSSSSFTPNNNTP V...... HiaNm 201 Hsf DVEKTRAATV KDVLNAGWNI KGAKTAGGNV ESVDLVSAYN NVEFITGDKN Hsf TLDVVLTAKE NGKTTEVKFT PKTSVIKEKD GKLFTGKENN DTNKVTSNTA Hsf TDNTDEGNGL VTAKAVIDAV NKAGWRVKTT TANGQNGDFA TVASGTNVTF HiaNm Hsf ESGDGTTASV TKDTNGNGIT VKYDAKVGDG LKFDSDKKIV ADTTALTVTG 401 Hsf GKVAEIAKED DKKKLVNAGD LVTALGNLSW KAKAEADTDG ALEGISKDQE HiaNm Hsf VKAGETVTFK AGKNLKVKQD GANFTYSLQD ALTGLTSITL GGTTNGGNDA HiaNm Hsf KTVINKDGLT ITPAGNGGTT GTNTISVTKD GIKAGNKAIT NVASGLRAYD HiaLKAYG HiaNm

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FIG.	
	551 600
Hsf	DANFDVLNNS ATDLNRHVED AYKGLLNLNE KNANKQPLVT DSTAATVGDL DANFNFTNNS IADAEKQVQE AYKGLLNLNE KNASDKLLVE DNTAATVGNL
Hia HiaNm	DANFNFTNNS IADAEKQVQE AYKGLLNLNE KNASDKLLVE DNTAATVGNL
птами	DI VQAI VAVI
	601 650
Hsf	RKLGWVVSTK NGTKEE.SNQ VKQAD.EVLF TGAGAATVTS KSENGKHTIT
Hia	RKLGWVLSSK NGTRNEKSQQ VKHAD.EVLF EGKGGVQVTS TSENGKHT
HiaNm	IVNSDK EGT.GEKEKV EENSDWAVYF NEKGVLT
	651 700
Hsf	VSVAETKADC GLEKDGDTIK LKVDNQNTDN VLTVGNNGTA VTKGGFETVK
Hia	
HiaNm	
	701 750
Hsf	TGATDADRGK VTVKDATAND ADKKVATVKD VATAINSAAT FVKTENLTTS
Hia HiaNm	
mann	
	751 800
Hsf	IDEDNPTDNG KDDALKAGDT LTFKAGKNLK VKRDGKNITF DLAKNLEVKT
Hia	ITF ALAKDLGVKT
HiaNm	
	801 850
Hsf	AKVSDTLTIG GNTPTGGTTA TPKVNITSTA DGLNFAKETA DASGSKNVYL
Hia	ATVSDTLTIG GGAAAGATT. TPKVNVTSTT DGLKFAKDAA GANG
HiaNm	SVGTEKLSFS ANGNKVNITSDT KGLNFAKETA GTNG
	900
	651
Hsf Hia	KGIATTLTEP SAGAKSSHVD LNVDATKKSN AASIEDVLKA GWNIQGNGNN
HiaNm	************

	901 950
Hsf	VDYVATYDTV NFTDDSTGTT TVTVTQKADG KGADVKIGAK TSVIKDHNGK
Hia	
HiaNm	
	951 1000
Hsf	LFTGKDLKDA NNGATVSEDD GKDTGTGLVT AKTVIDAVNK SGWRVTGEGA
Hia	
${\tt HiaNm}$	
	1001
We f	1001 1050 TAETGATAVN AGNAETVTSG TSVNFKNGNA TTATVSKDNG NINVKYDVNV
Hsf Hia	
HiaNm	
	1051 1100
Hsf	
Hia HiaNm	
UTANII	***************************************

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FIG. 6 cont'd 1101 1150 Hsf LNNLSWTAKA DKYADGESEG ETDQEVKAGD KVTFKAGKNL KVKOSEKDFT 1200 Hsf YSLQDTLTGL TSITLGGTAN GRNDTGTVIN KDGLTITLAN GAAAGTDASN Hsf GNTISVTKDG ISAGNKEITN VKSALKTYKD TQNTADETQD KEFHAAVKNA 1300 Hsf NEVEFVGKNG ATVSAKTDNN GKHTVTIDVA EAKVGDGLEK DTDGKIKLKV 1301 Hsf DNTDGNNLLT VDATKGASVA KGEFNAVTTD ATTAQGTNAN ERGKVVVKGS Hsf NGATATETDK KKVATVGDVA KAINDAATFV KVENDDSATI DDSPTDDGAN Hia Hsf DALKAGDTLT LKAGKNLKVK RDGKNITFAL ANDLSVKSAT VSDKLSLGTN Hsf GNKVNITSDT KGLNFAKDSK TGDDANIHLN GIASTLTDTL LNSGATTNLGVHLN GIGSTLTDTL VGSPATHIDG HiaNmVHLN GIGSTLTDTL LNTGATTNVT Hsf GNGITDNEKK RAASVKDVLN AGWNVRGVKP ASANNQVENI DFVATYDTVD Hia GDQSTHY..T RAASIKDVLN AGWNIKGVKA GSTTGQSENV DFVHTYDTVE Hianm NDNVTDDEKK RAASVKDVLN AGWNIKGVKP GTTA..SDNV DFVRTYDTVE 1600 1551 Hsf FVSGDKDTTS VTVESKDNGK RTEVKIGAKT SVIKDHNGKL FTGKELKDAN Hia FLSADTETTT VTVDSKENGK RTEVKIGAKT SVIKEKDGKL FTGKANKETN HiaNm FLSADTKTTT VNVESKDNGK KTEVKIGVKT SVIKEKDGKL VTGKD.KGEN Hsf NNGVTVTETD GKDEGNGLVT AKAVIDAVNK AGWRVKTTGA NGQNDD...F KVD.GANATE DADEGKGLVT AKDVIDAVNK TGWRIKTTDA NGQNGD...F

......GS STDEGEGLVT AKEVIDAVNK AGWRMKTTTA NGQTGQADKF

FIG. 6 cont'd

		-			
Hsf Hia HiaNm	1651 ATVASGTNVT ATVASGTNVT ETVTSGTNVT		VTNGTDG.IT	VKYNVKVADG VKYDAKVGDG VMYDVNVGDA	LKLDGDKIAA
Hsf Hia HiaNm		GKV KNANNPKGKV	ADVASTDEKK		LNSLSWTTTA
Hsf Hia HiaNm	AEADGGTLD.	ANSAGQEVKA GNASEQEVKA GNVSPSKGKM	GDKVTFKAGK	NLKVKQEGAN	FTYSLQDALT
Hsf Hia HiaNm	.GLTSITLGT	ANGGTGSEST GNNGAKT	EINKDGLTIT	PANGAGA	NNANTISVTK
Hsf Hia HiaNm	DGISAGGQSV	TNVVSGLKKF KNVVSGLKKF	GDANFDPLTS	SADNLTKQND	
Hsf Hia HiaNm	1901 EKGADNN.PT EKGTDKQTPV	VADNTAATVG VADNTAATVG	DLRGLGWVIS	ADKTTGGST.	1950 EYNAQVRNAN EYHDQVRNAN
Hsf Hia HiaNm	EVKFKSGNGI	NVSGKTLNGT NVSGKTVNGR	REITFELAKG	EVVKSNEFTV	2000 KNADGSETNL KETNGKETSL
Hsf Hia HiaNm	2001 VKVGDMYYSI VKVGDKYYSI	EDIDLTTGQP	KLKDGNTVAP	√ KYQDKGGKVV	2050 SANGSKTEVT SVTD.NTEAT
Hsf Hia HiaNm	ITNKGSGYV.	GNQVADAIAH HAIADAVQND 1	C SGFELGLADE	E ADAKRAFDD.	2100 S AKDKQLSKDK .KTKALSAGT
Hsf Hia HiaNm	TEIVNAHDK	V RFANGLNTK\	/ SAATVESTD	A NGDKVTTTF	2150 V KTDVELPLTQ V KTDVELPLTQ
Hsf Hia HiaNm	TYNTDANGK	K ITKVVKDGO	r KWYELNADG'	T ADMTKEVTLO	2200 G NVDANGKKVV G NVDSDGKKVV

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FIG. 6 cont'd

Hsf Hia	2201 KVTENGADKW KDNDGKW	YYTNADGAAD YHAKADGTAD	KTKGEVSNDK KTKGEVSNDK		
HiaNm	• • • • • • • • • • • • • • • • • • • •		• • • • • • • • • • • • • • • • • • • •	• • • • • • • • • • • • • • • • • • • •	• • • • • • • • • • • • • • • • • • • •
Hsf	2251 GVVIDNVANG	EISATSTDAI	NGSQLYAVAK	GVTNLAGQVN	2300 NLEGKVNKVG
Hia	GVVIDNVANG	DISATSTDAI	NGSQLYAVAK	GVTNLAGQVN	NLEGKVNKVG
${\tt HiaNm}$	VTNVA		QLKGVA.	Q	NLNNRIDNVD
	2301				2350
	2301				2330
Hsf	KRADAGTASA	LAASQLPQAT			AIGVSRISDN
Hsf Hia	KRADAGTASA KRADAGTASA	LAASQLPQAT	MPGKSMVAIA	GSSYQGQNGL	AIGVSRISDN AIGVSRISDN
	KRADAGTASA KRADAGTASA	LAASQLPQAT	MPGKSMVAIA	GSSYQGQNGL	AIGVSRISDN
Hia	KRADAGTASA KRADAGTASA GNARAGIAQA	LAASQLPQAT	MPGKSMVAIA LPGKSMMAIG	GSSYQGQNGL	AIGVSRISDN AIGVSRISDN
Hia HiaNm	KRADAGTASA KRADAGTASA GNARAGIAQA 2351	LAASQLPQAT IATAGLVQAY	MPGKSMVAIA LPGKSMMAIG 2378	GSSYQGQNGL	AIGVSRISDN AIGVSRISDN
Hia HiaNm Hsf	KRADAGTASA KRADAGTASA GNARAGIAQA 2351 GKVIIRLSGT	LAASQLPQAT IATAGLVQAY TNSQGKTGVA	MPGKSMVAIA LPGKSMMAIG 2378 AGVGYQW*	GSSYQGQNGL	AIGVSRISDN AIGVSRISDN
Hia HiaNm	KRADAGTASA KRADAGTASA GNARAGIAQA 2351 GKVIIRLSGT GKVIIRLSGT	LAASQLPQAT IATAGLVQAY	MPGKSMVAIA LPGKSMMAIG 2378 AGVGYQW* AGVGYQW*	GSSYQGQNGL	AIGVSRISDN AIGVSRISDN

FIG. 7

	1				5.0
	1				50
eg329	MNEILRIIWN	SALNAWVVVS		SATVKTAVLA	
pmc21	MNKIYRIIWN	SALNAWVVVS	ELTRNHTKRA	SATVKTAVLA	
HiaNm	MNKIYRIIWN	SALNAWVVVS	ELTRNHTKRA	SATVKTAVLA	TLLFATVQAS
h15	MNKIYRIIWN	SALNAWVVVS	ELTRNHTKRA	SATVATAVLA	TLLFATVQAN
BZ10	MNKISRIIWN	SALNAWVVVS	ELTRNHTKRA	SATVATAVLA	TLLFATVQAN
bz198	MNKIYRIIWN	SALNAWVVVS	ELTRNHTKRA	SATVATAVLA	TLLFATVQAN
eg327	MNKIYRIIWN	SALNAWVAVS	ELTRNHTKRA	SATVATAVLA	TLLFATVQAS
h38	MNKIYRIIWN	SALNAWVAVS	ELTRNHTKRA	SATVKTAVLA	TLLFATVOAN
h41	MNKIYRIIWN	SALNAWVAVS	ELTRNHTKRA	SATVKTAVLA	TLLFATVOAN
p20	MNKIYRIIWN	SALNAWVVVS	ELTRNHTKRA		TLLSATVOAN
P20					
	51				100
eg329	ANNE.EOEED	LYLDPVLRTV	NAU TANIGURE	GTGEKEKVEE	NSDWAVYFNE
	_	LYLDPVORTV		GTGEKEKVEE	NSDWAVYFNE
pmc21	ANNE EQEED			GTGEKEKVEE	NSDWAVYFNE
HiaNm	ANNERPRKKD	LYLDPVQRTV			
h15	ATDDDD	LYLEPVORTA		GTGEKE.GTE	DSNWAVYFDE
BZ10	ATDDDD		VVLSFRSDKE	GTGEKE.GTE	DSNWAVYFDE
bz198	ATDDDD	· 	VVLSFRSDKE	GTGEKE.GTE	DSNWAVYFDE
eg327	TTDDDD		VVLSFRSDKE	GTGEKE.VTE	DSNWGVYFDK
h38	ATDEDEE	EELEPVVRSA	LVLQFMIDKE	GNGENE.STG	NIGWSIYYDN
h41	ATDEDEE	EELESVQRS.	VVGSIQASME	GSVELETI	slsmtnds
p20	ATDTDED	EELESVARSA	LVLQFMIDKE	GNGEIE.STG	DIGWSIYYDD
_					
	101				150
eg329	KGVLTA.REI	TLKAGDNLKI	ко	NGTNFTYS	LKKDLTDLTS
pmc21	KGVLTA.REI	TLKAGDNLKI	ко	NGTNFTYS	LKKDLTDLTS
HiaNm	KGVLTA.REI	TLKAGDNLKI	ко	NGTNFTYS	LKKDLTDLTS
h15	KRVLKA.GAI	TLKAGDNLKI	KONTNENTNE	NTNDSSFTYS	LKKDLTDLTS
BZ10	KRVLKA.GAI	TLKAGDNLKI	KONTNENTNE	NTNDSSFTYS	LKKDLTDLTS
bz198	KRVLKA.GAI	TLKAGDNLKI	KONTNE	NTNDSSFTYS	LKKDLTDLTS
		TLKAGDNLKI	KONTNE	NTNASSETYS	LKKDLTDLTS
eg327	KGVLTA.GTI				LKKDLTDLTS
h38	HNTLHG.ATV			•	LKKDLTGLIN
h41	KEFVDPYIVV				
p20	HNTLHG.ATV	TLKAGDNLKI	KQ	SGKDFTYS	PVVPTVDDTTP
					200
	151				
eg329	VGTEKLSFSA	NGNKVNITSD			
pmc21	VGTEKLSFSA	. NGNKVNITSE			
HiaNm	VGTEKLSFSA	NGNKVNITSI			
h15	VETEKLSFGA	NGNKVNITSE	TKGLNFAKET	AGTNGDPTVH	
BZ10		NGNKVNITSI		AGTNGDPTVE	LNGIGSTLTD
bz198		NGNKVNITSI		AGTNGDPTVE	I LNGIGSTLTD
eg327		NSNKVNITSI		AETNGDTTV	I LNGIGSTLTD
h38		NGNKVNITSI			I LNGIGSTLTD
h41		NGKKVNIISI			
p20		NGNKVNITSI			-
p20	APIEVTOLGY	I MOMINAMETER	> 11/07H417H471		·

FIG. 7 cont'd

200	201				250
eg329	TLLNTGATTN		KKRAASVKDV		
pmc21	TLLNTGATTN		KKRAASVKDV		
HiaNm	TLLNTGATTN	VTNDNVTDDE		LNAGWNIKGV	
h15	TLLNTGATTN	VTNDNVTDDE	KKRAASVKDV	LNAGWNIKGV	
BZ10	TLLNTGATTN	VTNDNVTDDE		LNAGWNIKGV	
bz198	TLLNTGATTN	VTNDNVTDDE	KKRAASVKDV	LNAGWNIKGV	
eg327	TLLNTGATTN	VTNDNVTDDE	KKRAASVKDV	LNAGWNIKGV	
h38	TLLNTGATTN		KKRAASVKDV	LNAGWNIKGV	
h41	MLLNTGATTN	VTNDNVTDDE		LNAGWNIKGV	
p20	TLAGSSASHV	DAGNOSTHY.	.TRAASIKDV	LNAGWNIKGV	KTGSTTGQSE
	251				300
200	251	The section of the se	mm: All recurbit	CUUMETULLCA	
eg329	NVDFVRTYDT	VEFLSADTKT	TTVNVESKDN	GKKTEVKIGA GKKTEVKIGA	
pmc21	NVDFVRTYDT	VEFLSADTKT	TTVNVESKDN		
HiaNm	NVDFVRTYDT	VEFLSADTKT	TTVNVESKDN	GKKTEVKIGV	
h15	NVDFVRTYDT	VEFLSADTKT	TTVNVESKDN		KTSVIKEKDG
BZ10	NVDFVRTYDT	VEFLSADTKT	TTVNVESKDN		KTSVIKEKDG
bz198	NVDFVRTYDT	VEFLSADTKT	TTVNVESKDN		KTSVIKEKDG
eg327	NVDFVRTYDT	VEFLSADTKT	TTVNVESKDN		KTSVIKEKDG
h38	NVDFVHTYDT	VEFLSADTKT	TTVNVESKDN		KTSVIKEKDG
h41	NVDFVRTYDT	VEFLSADTKT	TTVNVESKDN		KTSVIKEKDG
p20	NVDFVRTYDT	VEFLSADTKT	TTVNVESKDN	GKRTEVKIGA	KTSVIKEKDG
	201				350
220	301 KLVTGKDKGE	NGSSTDEGEG	בחדעים אבייעדו	VNKAGWRMKT	TTANGOTGOA
eg329	•••	NGSSIDEGEG		VNKAGWRMKT	TTANGOTGOA
pmc21	KLVTGKDKGE	•••		VNKAGWRMKT	TTANGQTGQA
HiaNm	KLVTGKDKGE	NGSSTDEGEG		VNKAGWRMKT	TTANGQTGQA
h15	KLVTGKGKDE			VNKAGWRMKT	TTANGQTGQA
BZ10	KLVTGKGKGE				TTANGQTGQA
bz198	KLVTGKGKDE			VNKAGWRMKT	TTANGOTGOA
eg327	KLVTGKDKGE			VNKAGWRMKT	
h38	KLVTGKGKGE			VNKAGWRMKT	TTANGQTGQA
h41	KLVTGKGKGE			VNKAGWRMKT	
p20	KLVTGKGKGE	NGSSTDEGEG	LVTAKEVIDA	VNKAGWRMKT	TTANGQTGQA
	351				400
220	DKFETVTSGT	NVTFASGKGT	מסמתאפעייים י	NITVMYDVNV	GDALNVNQLQ
eg329	_				
pmc21	DKFETVTSGT				
HiaNm	DKFETVTSGT	_			
h15	DKFETVTSGT				
BZ10	DKFETVTSG				
bz198	DKFETVTSG		-		
eg327	DKFETVTSG				
h38	DKFETVTSG				
h41	DKFETVTSG				
p20	DKFETVTSG	r KVTFASGNG	TATVSKDDQO	MITAKIDAN/	/ GDALNVNQLQ

FIG. 7 cont'd

	401				450
eg329	NSGWNLDSKA		GNVSPSKGKM		NIEITRNGKN
pmc21	NSGWNLDSKA		GNVSPSKGKM	DETVNINAGN	NIEITRNGKN
HiaNm	NSGWNLDSKA		GNVSPSKGKM	DETVNINAGN	NIEITRNGKN
h15	NSGWNLDSKA		GNVSPSKGKM	DETVNINAGN	NIEITRNGKN
BZ10	NSGWNLDSKA		GNVSPSKGKM	DETVNINAGN	NIEITRNGKN
bz198	NSGWNLDSKA		GNVSPSKGKM	DETVNINAGN	NIEITRNGKN
eg327	NSGWNLDSKA		GNVSPSKGKM	DETVNINAGN	NIEITRNGKN
h38	NSGWNLDSKA		GNVSPSKGKM	DETVNINAGN	NIEITRNGKN
h41	NSGWNLDSKA		GNVSPSKGKM	DETVNINAGN	NIEITRNGKN
p20	NSGWNLDSKA	VAGSSGKVIS	GNVSPSKGKM	DETVNINAGN	NIEITRNGKN
	451				500
220		ECCUCIONON	DAPTLSVDGD	.ALNVGSKKD	NKPVRITNVA
eg329	IDIATSMTPO		DAPTLSVDGD	.ALNVGSKKD	
pmc21	IDIATSMTPQ IDIATSMTPQ		DAPTLSVDGD		NKEVRITNVA
HiaNm			DAPTLSVDDE		NKPVRITNVA
h15	IDIATSMTPQ		DAPTLSVDDE		NKEVRITNVA
BZ10	IDIATSMTPQ		DAPTLSVDDE	GALNVGSKDT	NKPVRITNVA
bz198	IDIATSMAPQ		DAPTLSVDDE		NKPVRITNVA
eg327	IDIATSMTPQ		DAPTLSVDDK		NKPVRITNVA
h38	IDIATSMTPQ		DAPTLSVDDE		NKPVRITNVA
h41	IDIATSMTPQ	FSSVSLGAGA			NKPVRITNVA
p20	IDIATSMTPQ	I 22 A 2 D GWGW	DALIESADDE	GALINVGSRDA	141/1 41/111111
	501				550
eg329	PGVKEGDVTN	VAQLKGVAQN	LNNRIDNVDG	NARAGIAQAI	ATAGLVQAYL
pmc21	PGVKEGDVTN	VAQLKGVAQN	LNNRIDNVDG	NARAGIAQAI	ATAGLVQAYL
HiaNm	PGVKEGDVTN		LNNRIDNVDG	NARAGIAQAI	ATAGLVQAYL
h15	PGVKEGDVTN		LNNRIDNVDG	NARAGIAQAI	ATAGLAQAYL
BZ10	PGVKEGDVTN		LNNRIDNVDG	NARAGIAQAI	ATAGLAQAYL
bz198	PGVKEGDVTN	VAOLKGVAON	LNNRIDNVDG	NARAGIAQAI	ATAGLVQAYL
eg327	PGVKEGDVTN	VAQLKGVAQN	LNNHIDNVDG	NARAGIAQAI	ATAGLVQAYL
h38	PGVKEGDVTN	VAQLKGVAQN	LNNRIDNVDG	NARAGIAQAI	ATAGLVQAYL
h41	PGVKEGDVTN		LNNRIDNVNG	NARAGIAQAI	ATAGLVQAYL
p20	PGVKEGDVTN	VAQLKGVAQN	LNNRIDNVNG	NARAGIAQAI	ATAGLAQAYL
•					500
	551				600
eg329	PGKSMMAIGG				
pmc21	PGKSMMAIGG				
HiaNm	PGKSMMAIGG		'		
h15	PGKSMMAIGG				
BZ10	PGKSMMAIGG				
bz198	PGKSMMAIGG				
eg327	PGKSMMAIGG				
h38	PGKSMMAIGG				
h41	PGKSMMAIG				
p20	PGKSMMAIG	GTYLGEAGY	A IGYSSISDTO	NWVIKGTAS	NSRGHFGTSA

FIG.	7 cont'd	
	601	
eg329	SVGYQW*	
pmc21	SVGYQW*	
HiaNm	SVGYQW*	
h15	SVGYQW*	
BZ10	SVGYQW*	
bz198	SVGYQW*	
eg327	SVGYQW*	
h38	SVGYQW*	
h41	SVGYQW*	
p20	SVGYOW*	

i

SEQUENCE LISTING

<pre><110> Peak, Ian R. (U.S. only) Jennings, Michael P. (U.S. only) Moxom, Edward R. (U.S. only) University of Queensland (except U.S.) Isis Innovations Limited (except U.S.)</pre>	
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cacgtcccag attcccgcct tcgcggggaa tgacgagatt ttaagttggg ggaatttatc 18	30
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aca cgc aac cac acc aaa cgc gcc tcc gca acc gtg aag acc gcc gta 38 Thr Arg Asn His Thr Lys Arg Ala Ser Ala Thr Val Lys Thr Ala Val 25 30 35	39
ttg gcg aca ctg ttg ttt gca acg gtt cag gca agt gct aac aat gaa 43 Leu Ala Thr Leu Leu Phe Ala Thr Val Gln Ala Ser Ala Asn Asn Glu 40 45 50	37
aga cca aga aag aaa gat tta tat tta gac ccc gta caa cgc act gtt 48 Arg Pro Arg Lys Lys Asp Leu Tyr Leu Asp Pro Val Gln Arg Thr Val 55 60 65 70	35
gcc gtg ttg ata gtc aat tcc gat aaa gaa ggc acg gga gaa aaa gaa 53 Ala Val Leu Ile Val Asn Ser Asp Lys Glu Gly Thr Gly Glu Lys Glu 75 80 85	33

ii

			Glu					Ala			ttc Phe		Glu			581
gta Val	cta Leu	aca Thr	90 gcc Ala	aga Arg	gaa Glu	atc Ile	acc Thr	95 ctc	aaa Lvs	gcc Ala	ggc Gly	gac	aac	ctg	aaa Luc	629
		105					110				ctg	115			,	677
Ile	Lys 120	Gln	Asn	Gly	Thr	Asn 125	Phe	Thr	Tyr	Ser	Leu 130	Lys	Lys	Asp	Leu	677
aca Thr 135	gat Asp	ctg Leu	acc Thr	agt Ser	gtt Val 140	gga Gly	act Thr	gaa Glu	aaa Lys	tta Leu 145	tcg Ser	ttt Phe	agc Ser	gca Ala	aac Asn 150	725
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											gtt Val					821
											acc Thr					869
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											gtc Val					1013
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gaa Glu	agc Ser	aaa Lys 265	gac Asp	aac Asn	ggc Gly	aag Lys	aaa Lys 270	acc Thr	gaa Glu	gtt Val	aaa Lys	atc Ile 275	ggt Gly	gtg Val	aag Lys	1109
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gca Ala	aaa Lys	gaa Glu	gtg Val	att Ile 315	gat Asp	gca Ala	gta Val	aac Asn	aag Lys 320	gct Ala	ggt Gly	tgg Trp	aga Arg	atg Met 325	aaa Lys	1253
aca Thr	aca Thr	acc Thr	gct Ala 330	aat Asn	ggt Gly	caa Gln	aca Thr	ggt Gly 335	caa Gln	gct Ala	gac Asp	aag Lys	ttt Phe 340	gaa Glu	acc Thr	1301

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iii

gtt Val	aca Thr	tca Ser 345	ggc Gly	aca Thr	aat Asn	gta Val	acc Thr 350	ttt Phe	gct Ala	agt Ser	ggt Gly	aaa Lys 355	ggt Gly	aca Thr	act Thr	1349
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gta Val 375	aat Asn	gtc Val	ggc Gly	gat Asp	gcc Ala 380	cta Leu	aac Asn	gtc Val	aat Asn	cag Gln 385	ctg Leu	caa Gln	aac Asn	agc Ser	ggt Gly 390	1445
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													acc Thr 420			1541
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													tcg Ser			1637
													gca Ala			1685
													aat Asn			1733
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tat Tyr 535	ttg Leu	ccc Pro	ggc Gly	aag Lys	agt Ser 540	atg Met	atg Met	gcg Ala	atc Ile	ggc Gly 545	ggc Gly	ggc Gly	act Thr	tat Tyr	cgc Arg 550	1925
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ggt Gly	gct Ala	tcc Ser 585	gca Ala	tct Ser	gtc Val	ggt Gly	tat Tyr 590	cag Gln	tgg Trp	taa	gggd	ottta	atc q	geet	gtctgc	2074

tgttgggaca ggcggaaggt ttgaagggaa gggtggcgat ttgccgcctg agacctttgc 2134

iv

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v

Val	Lys	Ile 275	Gly	Val	Lys	Thr	Ser 280	Val	Ile	Lys	Glu	Lys 285	Asp	Gly	Lys
Leu	Val 290	Thr	Gly	Lys	Asp	Lys 295	Gly	Glu	Asn	Gly	Ser 300	Ser	Thr	Asp	Glu
Gly 305	Glu	Gly	Leu	Val	Thr 310	Ala	Lys	Glu	Val	Ile 315	Asp	Ala	Val	Asn	Lys 320
Ala	Gly	Trp	Arg	Met 325	Lys	Thr	Thr	Thr	Ala 330	Asn	Gly	Gln	Thr	Gly 335	Gln
Ala	Asp	Lys	Phe 340	Glu	Thr	Val	Thr	Ser 345	Gly	Thr	Asn	Val	Thr 350	Phe	Ala
Ser	Gly	Lys 355	Gly	Thr	Thr	Ala	Thr 360	Val	Ser	Lys	Asp	Asp 365	Gln	Gly	Asn
Ile	Thr 370	Val	Met	Tyr	Asp	Val 375	Asn	Val	Gly	Asp	Ala 380	Leu	Asn	Val	Asn
Gln 385	Leu	Gln	Asn	Ser	Gly 390	Trp	Asn	Leu	Asp	Ser 395	Lys	Ala	Val	Ala	Gly 400
Ser	Ser	Gly	Lys	Val 405	Ile	Ser	Gly	Asn	Val 410	Ser	Pro	Ser	Lys	Gly 415	Lys
Met	Asp	Glu	Thr 420	Val	Asn	Ile	Asn	Ala 425	Gly	Asn	Asn	Ile	Glu 430	Ile	Thr
Arg	Asn	Gly 435	Lys	Asn	Ile	Asp	Ile 440	Ala	Thr	Ser	Met	Thr 445	Pro	Gln	Phe
Ser	Ser	Val	Ser	Leu	Gly	Ala 455	Gly	Ala	Asp	Ala	Pro 460	Thr	Leu	Ser	Val
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Asp 465	450 Gly	Asp	Ala	Leu	Asn 470		Gly	Ser	Lys	Lys 475		Asn	Lys	Pro	Val 480
465					470	Val				475	Asp				480
465 Arg	Gly	Thr	Asn	Val 485	470 Ala	Val Pro	Gly	Val	Lys 490	475 Glu	Asp Gly	Asp	Val	Thr 495	480 Asn
465 Arg Val	Gly	Thr Gln	Asn Leu 500	Val 485 Lys	470 Ala Gly	Val Pro Val	Gly Ala	Val Gln 505	Lys 490 Asn	475 Glu Leu	Asp Gly Asn	Asp Asn	Val Arg 510	Thr 495 Ile	480 Asn Asp
465 Arg Val Asn	Gly Ile Ala	Thr Gln Asp 515	Asn Leu 500 Gly	Val 485 Lys Asn	470 Ala Gly Ala	Val Pro Val Arg	Gly Ala Ala 520	Val Gln 505 Gly	Lys 490 Asn Ile	475 Glu Leu Ala	Asp Gly Asn Gln	Asp Asn Ala 525	Val Arg 510 Ile	Thr 495 Ile Ala	480 Asn Asp Thr
465 Arg Val Asn Ala	Gly Ile Ala Val	Thr Gln Asp 515 Leu	Asn Leu 500 Gly Val	Val 485 Lys Asn Gln	470 Ala Gly Ala Ala	Val Pro Val Arg Tyr 535	Gly Ala Ala 520 Leu	Val Gln 505 Gly Pro	Lys 490 Asn Ile Gly	475 Glu Leu Ala Lys	Asp Gly Asn Gln Ser 540	Asp Asn Ala 525 Met	Val Arg 510 Ile Met	Thr 495 Ile Ala Ala	480 Asn Asp Thr
Afg Val Asn Ala Gly 545	Gly Ile Ala Val Gly 530	Thr Gln Asp 515 Leu	Asn Leu 500 Gly Val	Val 485 Lys Asn Gln	A1a Gly Ala Ala Arg 550	Val Pro Val Arg Tyr 535 Gly	Gly Ala Ala 520 Leu Glu	Val Gln 505 Gly Pro	Lys 490 Asn Ile Gly	475 Glu Leu Ala Lys Tyr 555	Asp Gly Asn Gln Ser 540 Ala	Asp Asn Ala 525 Met	Val Arg 510 Ile Met	Thr 495 Ile Ala Ala Tyr	480 Asn Asp Thr Ile Ser 560

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<212> DNA

<213> Neisseria meningitidis

vi

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vii

	2> DI 3> Ne		eria	men	ingi	tidis	S									
	L> CI		(179	7)												
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				gat Asp												192
				ttg Leu												240
aaa Lys	gaa Glu	ggt Gly	aca Thr	gaa Glu 85	gat Asp	tca Ser	aat Asn	tgg Trp	gca Ala 90	gta Val	tat Tyr	ttc Phe	gac Asp	gag Glu 95	aaa Lys	288
				gcc Ala												336
				aac Asn												384
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acc Thr	gat Asp	acg Thr 195	ctg Leu	ctg Leu	aat Asn	acc Thr	gga Gly 200	gcg Ala	acc Thr	aca Thr	aac Asn	gta Val 205	acc Thr	aac Asn	gac Asp	624
aac Asn	gtt Val 210	acc Thr	gat Asp	gac Asp	gag Glu	aaa Lys 215	aaa Lys	cgt Arg	gcg Ala	gca Ala	agc Ser 220	gtt Val	aaa Lys	gac Asp	gta Val	672

tta aac gca ggc tgg aac att aaa ggc gtt aaa ccc ggt aca aca gct 720

viii

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agc Ser	gca Ala	gat Asp	acg Thr 260	aaa Lys	aca Thr	acg Thr	act Thr	gtt Val 265	aat Asn	gtg Val	gaa Glu	agc Ser	aaa Lys 270	gac Asp	aac Asn	816
ggc Gly	aag Lys	aga Arg 275	acc Thr	gaa Glu	gtt Val	aaa Lys	atc Ile 280	ggt Gly	gcg Ala	aag Lys	act Thr	tct Ser 285	gtt Val	att Ile	aaa Lys	864
gaa Glu	aaa Lys 290	gac Asp	ggt Gly	aag Lys	ttg Leu	gtt Val 295	act Thr	ggt Gly	aaa Lys	ggc Gly	aaa Lys 300	ggc Gly	gag Glu	aat Asn	ggt Gly	912
tct Ser 305	tct Ser	aca Thr	gac Asp	gaa Glu	ggc Gly 310	gaa Glu	ggc Gly	tta Leu	gtg Val	act Thr 315	gca Ala	aaa Lys	gaa Glu	gtg Val	att Ile 320	960
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ggt Gly	caa Gln	aca Thr	ggt Gly 340	caa Gln	gct Ala	gac Asp	aag Lys	ttt Phe 345	gaa Glu	acc Thr	gtt Val	aca Thr	tca Ser 350	ggc Gly	aca Thr	1056
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ccc Pro 465	act Thr	tta Leu	agc Ser	gtg Val	gat Asp 470	gac Asp	gag Glu	ggc Gly	gcg Ala	ttg Leu 475	aat Asn	gtc Val	ggc Gly	agc Ser	aag Lys 480	1440
gat Asp	gcc Ala	aac Asn	aaa Lys	ccc Pro	gtc Val	cgc Arg	att Ile	acc Thr	aat Asn	gtc Val	gcc Ala	ccg Pro	ggc Gly	gtt Val	aaa Lys	1488

Substitute Sheet (Rule 26) RO/AU

ix

4.8	35	490	495
		ctt aaa ggt gtg gcg Leu Lys Gly Val Ala 510	
		ggc aac gcg cgc gcg Gly Asn Ala Arg Ala 525	
		gct cag gcc tat ttg Ala Gln Ala Tyr Leu 540	
aag agt atg atg go Lys Ser Met Met Al 545	eg atc ggc ggc ggt la Ile Gly Gly Gly 550	act tat cgc ggc gaa Thr Tyr Arg Gly Glu 555	gcc ggt 1680 Ala Gly 560
Tyr Ala Ile Gly Ty		gac act ggg aat tgg Asp Thr Gly Asn Trp 570	
		ggt cat ttc ggt act Gly His Phe Gly Thr 590	
tot gto ggt tat ca Ser Val Gly Tyr G 595			1797
<210> 5 <211> 598 <212> PRT <213> Neisseria me	eningitidis		
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<211> 598 <212> PRT <213> Neisseria me <400> 5 Met Asn Lys Ile Se 1 Val Val Val Ser Gl	er Arg Ile Ile Trp 5 Lu Leu Thr Arg Asn 25	10 His Thr Lys Arg Ala	15 Ser Ala
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<pre><211> 598 <212> PRT <213> Neisseria me <400> 5 Met Asn Lys Ile Se 1 Val Val Val Ser Gl</pre>	er Arg Ile Ile Trp 5 Lu Leu Thr Arg Asn 25 La Val Leu Ala Thr 40 sp Asp Asp Asp Leu 55	His Thr Lys Arg Ala 30 Leu Leu Phe Ala Thr 45 Tyr Leu Glu Pro Val	15 Ser Ala Val Gln Gln Arg
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X

1	45					150					155					160
1	le	Thr	Ser	Asp	Thr 165	Lys	Gly	Leu	Asn	Phe 170	Ala	Lys	Glu	Thr	Ala 175	Gly
Tì	hr	Asn	Gly	Asp 180	Pro	Thr	Val	His	Leu 185	Asn	Gly	Ile	Gly	Ser 190	Thr	Leu
Tì	hr	Asp	Thr 195	Leu	Leu	Asn	Thr	Gly 200	Ala	Thr	Thr	Asn	Val 205	Thr	Asn	Asp
As	sn	Val 210	Thr	Asp	Asp	Glu	Lys 215	Lys	Arg	Ala	Ala	Ser 220	Val	Lys	Asp	Val
	eu 25	Asn	Ala	Gly	Trp	Asn 230	Ile	Lys	Gly	Val	Lys 235	Pro	Gly	Thr	Thr	Ala 240
Se	er	Asp	Asn	Val	Asp 245	Phe	Val	Arg	Thr	Tyr 250	Asp	Thr	Val	Glu	Phe 255	Leu
Se	er	Ala	Asp	Thr 260	Lys	Thr	Thr	Thr	Val 265	Asn	Val	Glu	Ser	Lys 270	Asp	Asn
G]	lу	Lys	Arg 275	Thr	Glu	Val	Lys	Ile 280	Gly	Ala	Lys	Thr	Ser 285	Val	Ile	Lys
G]	Lu	Lys 290	Asp	Gly	Lys	Leu	Val 295	Thr	Gly	Lys	Gly	Lys 300	Gly	Glu	Asn	Gly
Se 30		Ser	Thr	Asp	Glu	Gly 310	Glu	Gly	Leu	Val	Thr 315	Ala	Lys	Glu	Val	11e 320
As	зp	Ala	Val	Asn	Lys 325	Ala	Gly	Trp	Arg	Met 330	Lys	Thr	Thr	Thr	Ala 335	Asn
G1	Ly	Gln	Thr	Gly 340	Gln	Ala	Asp	Lys	Phe 345	Glu	Thr	Val	Thr	Ser 350	Gly	Thr
LΊ	/S	Val	Thr 355	Phe	Ala	Ser	Gly	Asn 360	Gly	Thr	Thr	Ala	Thr 365	Val	Ser	Lys
As	q	Asp 370	Gln	Gly	Asn	Ile	Thr 375	Val	Lys	Tyr	Asp	Val 380	Asn	Val	Gly	Asp
A1 38	la 35	Leu	Asn	Val	Asn	Gln 390	Leu	Gln	Asn	Ser	Gly 395	Trp	Asn	Leu	Asp	Ser 400
ΓŻ	/\$	Ala	Val	Ala	Gly 405	Ser	Ser	Gly	Lys	Val 410	Ile	Ser	Gly	Asn	Val 415	Ser
Pı	0	Ser	Lys	Gly 420	Lys	Met	Asp	Glu	Thr 425	Val	Asn	Ile	Asn	Ala 430	Gly	Asn
As	sn	Ile	Glu 435	Ile	Thr	Arg	Asn	Gly 440	Lys	Asn	Ile	Asp	Ile 445	Ala	Thr	Ser
Me	ŧ	Thr 450	Pro	Gln	Phe	Ser	Ser 455	Val	Ser	Leu	Gly	Ala 460	Gly	Ala	Asp	Ala
Pr 46	55	Thr	Leu	Ser	Val	Asp 470	Asp	Glu	Gly	Ala	Leu 475	Asn	Val	Gly	Ser	Lys 480
As	q	Ala	Asn	Lys	Pro 485	Val	Arg	Ile	Thr	Asn 490	Val	Ala	Pro	Gly	Val	Lys

хi

Glu	Gly	Asp	Val 500	Thr	Asn	Val	Ala	Gln 505	Leu	Lys	Gly	Val	Ala 510	Gln	Asn	
Leu	Asn	Asn 515	Arg	Ile	Asp	Asn	Val 520	Asp	Gly	Asn	Ala	Arg 525	Ala	Gly	Ile	
Ala	Gln 530	Ala	Ile	Ala	Thr	Ala 535	Gly	Leu	Ala	Gln	Ala 540	Tyr	Leu	Pro	Gly	
Lys 545	Ser	Met	Met	Ala	Ile 550	Gly	Gly	Gly	Thr	Tyr 555	Arg	Gly	Glu	Ala	Gly 560	
Tyr	Ala	Ile	Gly	Tyr 565	Ser	Ser	Ile	Ser	Asp 570	Thr	Gly	Asn	Trp	Val 575	Ile	
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	l> CI		(1785	5)												
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			tcc Ser 20													96
			acc Thr													144
gcg Ala	aat Asn 50	gct Ala	acc Thr	gat Asp	gac Asp	gac Asp 55	gat Asp	tta Leu	tat Tyr	tta Leu	gaa Glu 60	ccc Pro	gta Val	caa Gln	cgc Arg	192
			gtg Val													240
			aca Thr													288
aga Arg	gta Val	cta Leu	aaa Lys 100	gcc Ala	gga Gly	gca Ala	atc Ile	acc Thr 105	ctc Leu	aaa Lys	gcc Ala	ggc Gly	gac Asp 110	aac Asn	ctg Leu	336
aaa Lys	atc Ile	aaa Lys 115	caa Gln	aac Asn	acc Thr	aat Asn	gaa Glu 120	aac Asn	acc Thr	aat Asn	gac Asp	agt Ser 125	agc Ser	ttc Phe	acc Thr	384
tac	tcc	ctg	aaa	aaa	gac	ctc	aca	gat	ctg	acc	agt	gtt	gaa	act	gaa	432

xii

Tyr	Ser 130	Leu	Lys	Lys	Asp	Leu 135	Thr	Asp	Leu	Thr	Ser 140	Val	Glu	Thr	Glu	
aaa Lys 145	tta Leu	tcg Ser	ttt Phe	ggc Gly	gca Ala 150	aac Asn	ggt Gly	aat Asn	aaa Lys	gtc Val 155	aac Asn	atc Ile	aca Thr	agc Ser	gac Asp 160	480
acc Thr	aaa Lys	ggc Gly	ttg Leu	aat Asn 165	ttt Phe	gcg Ala	aaa Lys	gaa Glu	acg Thr 170	gct Ala	ggg Gly	acg Thr	aac Asn	ggc Gly 175	gac Asp	528
ccc Pro	acg Thr	gtt Val	cat His 180	ctg Leu	aac Asn	ggt Gly	atc Ile	ggt Gly 185	tcg Ser	act Thr	ttg Leu	acc Thr	gat Asp 190	acg Thr	ctg Leu	576
ctg Leu	aat Asn	acc Thr 195	gga Gly	gcg Ala	acc Thr	aca Thr	aac Asn 200	gta Val	acc Thr	aac Asn	gac Asp	aac Asn 205	gtt Val	acc Thr	gat Asp	624
gac Asp	gag Glu 210	aaa Lys	aaa Lys	cgt Arg	gcg Ala	gca Ala 215	agc Ser	gtt Val	aaa Lys	gac Asp	gta Val 220	tta Leu	aac Asn	gca Ala	ggc Gly	672
tgg Trp 225	aac Asn	att Ile	aaa Lys	ggc Gly	gtt Val 230	aaa Lys	ccc Pro	ggt Gly	aca Thr	aca Thr 235	gct Ala	tcc Ser	gat Asp	aac Asn	gtt Val 240	720
gat Asp	ttc Phe	gtc Val	cgc Arg	act Thr 245	tac Tyr	gac Asp	aca Thr	gtc Val	gag Glu 250	ttc Phe	ttg Leu	agc Ser	gca Ala	gat Asp 255	acg Thr	768
aaa Lys	aca Thr	acg Thr	act Thr 260	gtt Val	aat Asn	gtg Val	gaa Glu	agc Ser 265	aaa Lys	gac Asp	aac Asn	ggc Gly	aag Lys 270	aaa Lys	acc Thr	816
gaa Glu	gtt Val	aaa Lys 275	atc Ile	ggt Gly	gcg Ala	aag Lys	act Thr 280	tct Ser	gtt Val	att Ile	aaa Lys	gaa Glu 285	aaa Lys	gac Asp	ggt Gly	864
aag Lys	ttg Leu 290	gtt Val	act Thr	ggt Gly	aaa Lys	ggc Gly 295	aaa Lys	gac Asp	gag Glu	aat Asn	ggt Gly 300	tct Ser	tct Ser	aca Thr	gac Asp	912
gaa Glu 305	ggc Gly	gaa Glu	ggc Gly	tta Leu	gtg Val 310	act Thr	gca Ala	aaa Lys	gaa Glu	gtg Val 315	att Ile	gat Asp	gca Ala	gta Val	aac Asn 320	960
aag Lys	gct Ala	ggt Gly	tgg Trp	aga Arg 325	atg Met	aaa Lys	aca Thr	aca Thr	acc Thr 330	gct Ala	aat Asn	ggt Gly	caa Gln	aca Thr 335	ggt Gly	1008
caa Gln	gct Ala	gac Asp	aag Lys 340	ttt Phe	gaa Glu	acc Thr	gtt Val	aca Thr 345	tca Ser	ggc Gly	aca Thr	aat Asn	gta Val 350	acc Thr	ttt Phe	1056
gct Ala	agt Ser	ggt Gly 355	aaa Lys	ggt Gly	aca Thr	act Thr	gcg Ala 360	act Thr	gta Val	agt Ser	aaa Lys	gat Asp 365	gat Asp	caa Gln	ggc Gly	1104
aac Asn	atc Ile 370	act Thr	gtt Val	aag Lys	tat Tyr	gat Asp 375	gta Val	aat Asn	gtc Val	ggc Gly	gat Asp 380	gcc Ala	cta Leu	aac Asn	gtc Val	1152
aat Asn	cag Gln	ctg Leu	caa Gln	aac Asn	agc Ser	ggt Gly	tgg Trp	aat Asn	ttg Leu	gat Asp	tcc Ser	aaa Lys	gcg Ala	gtt Val	gca Ala	1200

xiii

385				390					395					400	
ggt to Gly Se	ct tc er Se	g ggc r Gly	aaa Lys 405	gtc Val	atc Ile	agc Ser	ggc Gly	aat Asn 410	gtt Val	tcg Ser	ccg Pro	agc Ser	aag Lys 415	gga Gly	1248
aag at Lys Me	tg ga et As	t gaa p Glu 420	acc Thr	gtc Val	aac Asn	att Ile	aat Asn 425	gcc Ala	ggc Gly	aac Asn	aac Asn	atc Ile 430	gag Glu	att Ile	1296
acc co	gc aa rg As: 43	n Gly	aaa Lys	aat Asn	atc Ile	gac Asp 440	atc Ile	gcc Ala	act Thr	tcg Ser	atg Met 445	gcg Ala	ccg Pro	cag Gln	1344
ttt to Phe Se 45	cc ag er Se 50	c gtt r Val	tcg Ser	ctc Leu	ggt Gly 455	gcg Ala	G]Å ååå	gcg Ala	gat Asp	gcg Ala 460	ccc Pro	act Thr	ttg Leu	agc Ser	1392
gtg ga Val As 465	at gad sp Asj	c gag p Glu	ggc Gly	gcg Ala 470	ttg Leu	aat Asn	gtc Val	Gly Gly	agc Ser 475	aag Lys	gat Asp	acc Thr	aac Asn	aaa Lys 480	1440
ccc gt Pro Va	tc cg	c att g Ile	acc Thr 485	aat Asn	gtc Val	gcc Ala	ccg Pro	ggc Gly 490	gtt Val	aaa Lys	gag Glu	ggg Gly	gat Asp 495	gtt Val	1488
aca aa Thr As															1536
atc ga Ile As	ac aa sp Asi 51	n Val	gac Asp	ggc Gly	aac Asn	gcg Ala 520	cgt Arg	gcg Ala	ggc Gly	atc Ile	gcc Ala 525	caa Gln	gcg Ala	att Ile	1584
gca ac Ala Th 53	cc gca nr Ala 30	a ggt a Gly	cta Leu	gtt Val	cag Gln 535	gcg Ala	tat Tyr	ctg Leu	ccc Pro	ggc Gly 540	aag Lys	agt Ser	atg Met	atg Met	1632
gcg at Ala Il 545															1680
tac to Tyr Se	ca agi er Se:	t att	tcc Ser 565	gac Asp	ggc Gly	gga Gly	aat Asn	tgg Trp 570	att Ile	atc Ile	aaa Lys	ggc Gly	acg Thr 575	gct Ala	1728
tcc gg Ser Gl	gc aat ly Asi	t tcg n Ser 580	cgc Arg	ggc Gly	cat His	ttc Phe	ggt Gly 585	gct Ala	tcc Ser	gca Ala	tct Ser	gtc Val 590	ggt Gly	tat Tyr	1776
caa to Gln Tr															1785
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Val Va	al Val	. Ser	Glu	Leu	Thr	Arg	Asn	His	Thr	Lys	Arg	Ala	Ser	Ala	

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			20					25					30		
Thr	Val	Ala 35	Thr	Ala	Val	Leu	Ala 40	Thr	Leu	Leu	Phe	Ala 45	Thr	Val	Gln
Ala	Asn 50	Ala	Thr	Asp	Asp	Asp 55	Asp	Leu	Tyr	Leu	Glu 60	Pro	Val	Gln	Arg
Thr 65	Ala	Val	Val	Leu	Ser 70	Phe	Arg	Ser	Asp	Lys 75	Glu	Gly	Thr	Gly	Glu 80
Lys	Glu	Gly	Thr	Glu 85	Asp	Ser	Asn	Trp	Ala 90	Val	Tyr	Phe	Asp	Glu 95	Lys
Arg	Val	Leu	Lys 100	Ala	Gly	Ala	Ile	Thr 105	Leu	Lys	Ala	Gly	Asp 110	Asn	Leu
Lys	Ile	Lys 115	Gln	Asn	Thr	Asn	Glu 120	Asn	Thr	Asn	Asp	Ser 125	Ser	Phe	Thr
Tyr	Ser 130	Leu	Lys	Lys	Asp	Leu 135	Thr	Asp	Leu	Thr	Ser 140	Val	Glu	Thr	Glu
Lys 145	Leu	Ser	Phe	Gly	Ala 150	Asn	Gly	Asn	Lys	Val 155	Asn	Ile	Thr	Ser	Asp 160
Thr	Lys	Gly	Leu	Asn 165	Phe	Ala	Lys	Glu	Thr 170	Ala	Gly	Thr	Asn	Gly 175	Asp
Pro	Thr	Val	His 180	Leu	Asn	Gly	Ile	Gly 185	Ser	Thr	Leu	Thr	Asp 190	Thr	Leu
Leu	Asn	Thr 195	Gly	Ala	Thr	Thr	Asn 200	Val	Thr	Asn	Asp	Asn 205	Val	Thr	Asp
Asp	Glu 210	Lys	Lys	Arg	Ala	Ala 215	Ser	Val	Lys	Asp	Val 220	Leu	Asn	Ala	Gly
Trp 225	Asn	Ile	Lys	Gly	Val 230	Lys	Pro	Gly	Thr	Thr 235	Ala	Ser	Asp	Asn	Val 240
Asp	Phe	Val	Arg	Thr 245	Tyr	Asp	Thr	Val	Glu 250	Phe	Leu	Ser	Ala	Asp 255	Thr
Lys	Thr	Thr	Thr 260	Val	Asn	Val	Glu	Ser 265	Lys	Asp	Asn	Gly	Lys 270	Lys	Thr
Glu	Val	Lys 275	Ile	Gly	Ala	Lys	Thr 280	Ser	Val	Ile	Lys	Glu 285	Lys	Asp	Gly
Lys	Leu 290	Val	Thr	Gly	Lys	Gly 295	Lys	Asp	Glu	Asn	Gly 300	Ser	Ser	Thr	Asp
Glu 305	Gly	Glu	Gly	Leu	Val 310	Thr	Ala	Lys	Glu	Val 315	Ile	Asp	Ala	Val	Asn 320
Lys	Ala	Gly	Trp	Arg 325	Met	Lys	Thr	Thr	Thr 330	Ala	Asn	Gly	Gln	Thr 335	Gly
Gln	Ala	Asp	Lys 340	Phe	Glu	Thr	Val	Thr 345	Ser	Gly	Thr	Asn	Val 350	Thr	Phe
Ala	Ser	Gly 355	Lys	Gly	Thr	Thr	Ala 360	Thr	Val	Ser	Lys	Asp 365	Asp	Gln	Gly

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Asn	Ile 370	Thr	Val	Lys	Tyr	Asp 375	Val	Asn	Val	Gly	Asp 380	Ala	Leu	Asn	Val	
Asn 385	Gln	Leu	Gln	Asn	Ser 390	Gly	Trp	Asn	Leu	Asp 395	Ser	Lys	Ala	Val	Ala 400	
Gly	Ser	Ser	Gly	Lys 405	Val	Ile	Ser	Gly	Asn 410	Val	Ser.	Pro	Ser	Lys 415	Gly	
Lys	Met	Asp	Glu 420	Thr	Val	Asn	Ile	Asn 425	Ala	Gly	Asn	Asn	Ile 430	Glu	Ile	
Thr	Arg	Asn 435	Gly	Lys	Asn	Ile	Asp 440	Ile	Ala	Thr	Ser	Met 445	Ala	Pro	Gln	
Phe	Ser 450	Ser	Val	Ser	Leu	Gly 455	Ala	Gly	Ala	Asp	Ala 460	Pro	Thr	Leu	Ser	
Val 465	Asp	Asp	Glu	Gly	Ala 470	Leu	Asn	Val	Gly	Ser 475	Lys	Asp	Thr	Asn	Lys 480	
Pro	Val	Arg	Ile	Thr 485	Asn	Val	Ala	Pro	Gly 490	Val	Lys	Glu	Gly	Asp 495	Val	
Thr	Asn	Val	Ala 500	Gln	Leu	Lys	Gly	Val 505	Ala	Gln	Asn	Leu	Asn 510	Asn	Arg	
Ile	Asp	Asn 515	Val	Asp	Gly	Asn	Ala 520	Arg	Ala	Gly	Ile	Ala 525	Gln	Ala	Ile	
Ala	Thr 530	Ala	Gly	Leu	Val	Gln 535	Ala	Tyr	Leu	Pro	Gly 540	Lys	Ser	Met	Met	
Ala 545	Ile	Gly	Gly	Asp	Thr 550	Tyr	Arg	Gly	Glu	Ala 555	Gly	Tyr	Ala	Ile	Gly 560	
Tyr	Ser	Ser	Ile	Ser 565	Asp	Gly	Gly	Asn	Trp 570	Ile	Ile	Lys	Gly	Thr 575	Ala	
Ser	Gly	Asn	Ser 580	Arg	Gly	His	Phe	Gly 585	Ala	Ser	Ala	Ser	Val 590	Gly	Tyr	
Gln	Trp															
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	.> CE	os L)(1785	5)												
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gtc Val	gcc Ala	gta Val	tcc Ser 20	gag Glu	ctc Leu	aca Thr	cgc Arg	aac Asn 25	cac His	acc Thr	aaa Lys	cgc Arg	gcc Ala 30	tcc Ser	gca Ala	96
acc Thr	gtg Val	gcg Ala	acc Thr	gcc Ala	gta Val	ttg Leu	gcg Ala	aca Thr	ctg Leu	ttg Leu	ttt Phe	gca Ala	acg Thr	gtt Val	cag Gln	14

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								-								
		35					40					45				
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act Thr 65	gct Ala	gtc Val	gtg Val	ttg Leu	agc Ser 70	ttc Phe	cgt Arg	tcc Ser	gat Asp	aaa Lys 75	gaa Glu	ggc Gly	acg Thr	gga Gly	gaa Glu 80	240
aaa Lys	gaa Glu	gtt Val	aca Thr	gaa Glu 85	gat Asp	tca Ser	aat Asn	tgg Trp	gga Gly 90	gta Val	tat Tyr	ttc Phe	gac Asp	aag Lys 95	aaa Lys	288
gga Gly	gta Val	cta Leu	aca Thr 100	gcc Ala	gga Gly	aca Thr	atc Ile	acc Thr 105	ctc Leu	aaa Lys	gcc Ala	ggc Gly	gac Asp 110	aac Asn	ctg Leu	336
aaa Lys	atc Ile	aaa Lys 115	caa Gln	aac Asn	acc Thr	aat Asn	gaa Glu 120	aac Asn	acc Thr	aat Asn	gcc Ala	agt Ser 125	agc Ser	ttc Phe	acc Thr	384
tac Tyr	tcg Ser 130	ctg Leu	aaa Lys	aaa Lys	gac Asp	ctc Leu 135	aca Thr	gat Asp	ctg Leu	acc Thr	agt Ser 140	gtt Val	gga Gly	act Thr	gaa Glu	432
aaa Lys 145	tta Leu	tcg Ser	ttt Phe	agc Ser	gca Ala 150	aac Asn	agc Ser	aat Asn	aaa Lys	gtc Val 155	aac Asn	atc Ile	aca Thr	agc Ser	gac Asp 160	480
acc Thr	aaa Lys	ggc Gly	ttg Leu	aat Asn 165	ttc Phe	gcg Ala	aaa Lys	aaa Lys	acg Thr 170	gct Ala	gag Glu	acc Thr	aac Asn	ggc Gly 175	gac Asp	528
acc Thr	acg Thr	gtt Val	cat His 180	ctg Leu	aac Asn	ggt Gly	atc Ile	ggt Gly 185	tcg Ser	act Thr	ttg Leu	acc Thr	gat Asp 190	acg Thr	ctg Leu	576
ctg Leu	aat Asn	acc Thr 195	gga Gly	gcg Ala	acc Thr	aca Thr	aac Asn 200	gta Val	acc Thr	aac Asn	gac Asp	aac Asn 205	gtt Val	acc Thr	gat Asp	624
gac Asp	gag Glu 210	aaa Lys	aaa Lys	cgt Arg	gcg Ala	gca Ala 215	agc Ser	gtt Val	aaa Lys	gac Asp	gta Val 220	tta Leu	aac Asn	gca Ala	ggc Gly	672
tgg Trp 225	aac Asn	att Ile	aaa Lys	ggc Gly	gtt Val 230	aaa Lys	ccc Pro	ggt Gly	aca Thr	aca Thr 235	gct Ala	tcc Ser	gat Asp	aac Asn	gtt Val 240	720
gat Asp	ttc Phe	gtc Val	cgc Arg	act Thr 245	tac Tyr	gac Asp	aca Thr	gtc Val	gag Glu 250	ttc Phe	ttg Leu	agc Ser	gca Ala	gat Asp 255	acg Thr	768
aaa Lys	aca Thr	acg Thr	act Thr 260	gtt Val	aat Asn	gtg Val	gaa Glu	agc Ser 265	aaa Lys	gac Asp	aac Asn	ggc Gly	aag Lys 270	aga Arg	acc Thr	816
gaa Glu	gtt Val	aaa Lys 275	atc Ile	ggt Gly	gcg Ala	aag Lys	act Thr 280	tct Ser	gtt Val	atc Ile	aaa Lys	gaa Glu 285	aaa Lys	gac Asp	ggt Gly	864
aag Lys	ttg Leu 290	gtt Val	act Thr	ggt Gly	aaa Lys	gac Asp 295	aaa Lys	ggc Gly	gag Glu	aat Asn	gat Asp 300	tct Ser	tct Ser	aca Thr	gac Asp	912

Substitute Sheet (Rule 26) RO/AU

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			ggc Gly													960
aag Lys	gct Ala	ggt Gly	tgg Trp	aga Arg 325	atg Met	aaa Lys	aca Thr	aca Thr	acc Thr 330	gct Ala	aat Asn	ggt Gly	caa Gln	aca Thr 335	ggt Gly	1008
			aag Lys 340													1056
			aaa Lys													1104
aac Asn	atc Ile 370	act Thr	gtt Val	atg Met	tat Tyr	gat Asp 375	gta Val	aat Asn	gtc Val	ggc Gly	gat Asp 380	gcc Ala	cta Leu	aac Asn	gtc Val	1152
		_	caa Gln		-				-	-				-	_	1200
			ggc Gly													1248
			gaa Glu 420													1296
			ggc Gly													1344
			gtt Val													1392
			gag Glu													1440
ccc Pro	gtc Val	cgc Arg	att Ile	acc Thr 485	Asn	gtc Val	gcc Ala	Pro	ggc Gly 490	Val	aaa Lys	gag Glu	ggg Gly	gat Asp 495	gtt Val	1488
			gca Ala 500													1536
			gtg Val													1584
gca Ala	acc Thr 530	gca Ala	ggt Gly	ctg Leu	gtt Val	cag Gln 535	gcg Ala	tat Tyr	ctg Leu	ccc Pro	ggc Gly 540	aag Lys	agt Ser	atg Met	atg Met	1632
gcg Ala 545	atc Ile	ggc Gly	ggc Gly	ggc Gly	act Thr 550	tat Tyr	cgc Arg	ggc Gly	gaa Glu	gcc Ala 555	ggt Gly	tat Tyr	gcc Ala	atc Ile	ggc Gly 560	1680

xviii

tac tca agc att tcc gac ggc gga aat tgg att atc aaa ggc acg gct Tyr Ser Ser Ile Ser Asp Gly Gly Asn Trp Ile Ile Lys Gly Thr Ala 565 570 575	1728
tcc ggc aat tcg cgc ggc cat ttc ggt gct tcc gca tct gtc ggt tat Ser Gly Asn Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr 580 585 590	1776
cag tgg taa Gln Trp 595	1785
<210> 9 <211> 594 <212> PRT <213> Neisseria meningitidis	
<400> 9	
Met Asn Lys Ile Tyr Arg Ile Ile Trp Asn Ser Ala Leu Asn Ala Trp 1 5 10 15	
Val Ala Val Ser Glu Leu Thr Arg Asn His Thr Lys Arg Ala Ser Ala 20 25 30	
Thr Val Ala Thr Ala Val Leu Ala Thr Leu Leu Phe Ala Thr Val Gln 35 40 45	
Ala Ser Thr Thr Asp Asp Asp Leu Tyr Leu Glu Pro Val Gln Arg 50 60	
Thr Ala Val Val Leu Ser Phe Arg Ser Asp Lys Glu Gly Thr Gly Glu 65 70 75 80	
Lys Glu Val Thr Glu Asp Ser Asn Trp Gly Val Tyr Phe Asp Lys Lys 85 90 95	
Gly Val Leu Thr Ala Gly Thr Ile Thr Leu Lys Ala Gly Asp Asn Leu 100 105 110	
Lys Ile Lys Gln Asn Thr Asn Glu Asn Thr Asn Ala Ser Ser Phe Thr 115 120 125	
Tyr Ser Leu Lys Lys Asp Leu Thr Asp Leu Thr Ser Val Gly Thr Glu 130 135 140	
Lys Leu Ser Phe Ser Ala Asn Ser Asn Lys Val Asn Ile Thr Ser Asp 145 150 155 160	
Thr Lys Gly Leu Asn Phe Ala Lys Lys Thr Ala Glu Thr Asn Gly Asp 165 170 175	
Thr Thr Val His Leu Asn Gly Ile Gly Ser Thr Leu Thr Asp Thr Leu 180 185 190	
Leu Asn Thr Gly Ala Thr Thr Asn Val Thr Asn Asp Asn Val Thr Asp 195 200 205	
Asp Glu Lys Lys Arg Ala Ala Ser Val Lys Asp Val Leu Asn Ala Gly 210 215 220	
Trp Asn Ile Lys Gly Val Lys Pro Gly Thr Thr Ala Ser Asp Asn Val 225 230 235 240	
Asp Phe Val Arg Thr Tyr Asp Thr Val Glu Phe Leu Ser Ala Asp Thr	

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				245					250					255	
Lys	Thr	Thr	Thr 260	Val	Asn	Val	Glu	Ser 265	Lys	Asp	Asn	Gly	Lys 270	Arg	Thr
Glu	Val	Lys 275	Ile	Gly	Ala	Lys	Thr 280	Ser	Val	Ile	Lys	Glu 285	Lys	Asp	Gly
Lys	Leu 290	Val	Thr	Gly	Lys	Asp 295	Lys	Gly	Glu	Asn	Asp 300	Ser	Ser	Thr	Asp
Lys 305	Gly	Glu	Gly	Leu	Val 310	Thr	Ala	Lys	Glu	Val 315	Ile	Asp	Ala	Val	Asn 320
Lys	Ala	Gly	Trp	Arg 325	Met	Lys	Thr	Thr	Thr 330	Ala	Asn	Gly	Gln	Thr 335	Gly
Gln	Ala	Asp	Lys 340	Phe	Glu	Thr	Val	Thr 345	Ser	Gly	Thr	Asn	Val 350	Thr	Phe
Ala	Ser	Gly 355	Lys	Gly	Thr	Thr	Ala 360	Thr	Val	Ser	Lys	Asp 365	Asp	Gln	Gly
Asn	Ile 370	Thr	Val	Met	Tyr	Asp 375	Val	Asn	Val	Gly	Asp 380	Ala	Leu	Asn	Val
Asn 385	Gln	Leu	Gln	Asn	Ser 390	Gly	Trp	Asn	Leu	Asp 395	Ser	Lys	Ala	Val	Ala 400
Gly	Ser	Ser	Gly	Lys 405	Val	Ile	Ser	Gly	Asn 410	Val	Ser	Pro	Ser	Lys 415	Gly
Lys	Met	Asp	Glu 420	Thr	Val	Asn	Ile	Asn 425	Ala	Gly	Asn	Asn	Ile 430	Glu	Ile
Thr	Arg	Asn 435	Gly	Lys	Asn	Ile	Asp 440	Ile	Ala	Thr	Ser	Met 445	Thr	Pro	Gln
Phe	Ser 450	Ser	Val	Ser	Leu	Gly 455	Ala	Gly	Ala	Asp	Ala 460	Pro	Thr	Leu	Ser
Val 465	Asp	Asp	Glu	Gly	Ala 470	Leu	Asn	Val	Gly	Ser 475	Lys	Asp	Ala	Asn	Lys 480
Pro	Val	Arg	Ile	Thr 485	Asn	Val	Ala	Pro	Gly 490	Val	Lys	Glu	Gly	Asp 495	Val
Thr	Asn	Val	Ala 500	Gln	Leu	Lys	Gly	Val 505	Ala	Gln	Asn	Leu	Asn 510	Asn	His
Ile	Asp	Asn 515	Val	Asp	Gly	Asn	Ala 520	Arg	Ala	Gly	Ile	Ala 525	Gln	Ala	Ile
Ala	Thr 530	Ala	Gly	Leu	Val	Gln 535	Ala	Tyr	Leu	Pro	Gly 540	Lys	Ser	Met	Met
Ala 545	Ile	Gly	Gly	Gly	Thr 550	Tyr	Arg	Gly	Glu	Ala 555	Gly	Tyr	Ala	Ile	Gly 560
Tyr	Ser	Ser	Ile	Ser 565	Asp	Gly	Gly	Asn	Trp 570	Ile	Ile	Lys	Gly	Thr 575	Ala
Ser	Gly	Asn	Ser 580	Arg	Gly	His	Phe	Gly 585	Ala	Ser	Ala	Ser	Val 590	Gly	Tyr

Substitute Sheet UA/OA (82 sluA)

624	ern ded	dse dsy	tap qeA	302 Трк ЗСС	get get	aac Asn	gac Asp	aac Asn	200 Тут 3сс	gta Val	ysu ysu	вса Тћг	усс Трх	gcg Ala 291	ejl das	дук Тук
949	taa Asn	ren c£d	190 гел сғд	gcd Thr	jap qaA	ург Трх	nəq ffd	асt Тћг 185	ger fcd	стл ааг	att Ile	gγλ ādr	390 Yan 180	cfd cfd	cat His	gtt Val
228	дуц чсд	Jys Thr 3CC	gac gac	gγλ ddc	sac Asn	цух чсд	710 C77 ddd	gct Ala	црк зсд	gyn Gyn	гла	gcg 818 291	ъре сее	taa neA	nəq Ted	Cγλ ddc
087	ges Lys	эсс	gac Asp	ser sgc	дуц Туц	atc 116 125	aac Asn	gtc Val	saa Lys	aat Asn	120 Gγλ ddc	aac Asn	gcs Ala	ger agc	₽Vd ∓∓∓	142 261 £cd
432	tta Leu	гва Гуѕ	ges	трк Трк	740 GJ\ dds	gtt gtt	sgt	дух Тух	ren c£d	tap qeA ZEI	вса Тћг	ctc	ysp dsc	ааа Гуѕ	130 Гуѕ	ctg Leu
384	ger	tac Tyr	дук ЗСС	152 bye ffc	ysu ysu	аса Тћг	eTy ggc	aac Asn	150 CJu C99	гуs	atc Ile	гуз аза	ren crd	aac Asn 211	gac gac	ejl ddc
988		груз														
588	tat Tyr	ste Lav 26	sce Ala	Lrp rdd	dsA qsA	tca	tss nsA 06	ggg Qgg	gg n	gta Val	гуs ааа	989 67 <i>n</i> 82	ваа Lys	dss GJ <i>n</i>	СŢЛ dds	ург Трг
240	80 CJN ddc	два СТл	ааа Гуѕ	jap qaA	tcc	tas neÆ ∂Γ	gtc Val	ata Ile	ren ttd	∆gj ∂¢∂	occ ala 07	gtt Val	дрк Трк	yrd cdc	cta Leu	gtg Val 85
192	ccc	gac	tta Leu	tat Tyr	tta Leu 09	тър qsА	ggn Gjn	gyn dys	сяя Сяя	22 032 032	gaa GTu	tss nsA	aac Asn	dct Ala	agt Ser 50	дсэ УГЭ
PPT	eyn csd	grt Val	gcd Thr	sop sIA 24	₽¥4 ₽₽₽	rfd ren	crd	дук Дук	gcg Ala 01	ren ççd	gta Val	gcc Ala	цук чсс	32 Pys 32	gtg Val	асс Тћг
96	gcs Ala	ser	oop sia 0£	yrd cdc	aaa Lys	дук Дук	csc	эвв пеА ЗS	cdc	дук Зся	ctc	dag G1n	20 Ser Lcc	gta Val	gtt gtt	grc
8 Þ	rad Lad	occ Ala 21	tss nsA	ctc	gcc	ger	taa naA 01	£ãã	att Ile	atc Ile	yzd cdc	paa Leu S	ata Ile	dsa)> 1(38C Asn	ard
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7392	dst Asp	Ag J Ag A	261 9dc	red Leu	асt Тћг 091	Pro ccc	yys aca	dst Asp	A79 aca	422 CJ X ddd	gcg gcg	ejl ddc	ctc Leu	2er Fcd	420 A97 dec	26r 9dc
7344	zec Ser	₽V4 ∓∓∓	eyn csd	445 Ero ccd	дух Тух	atg Met	ger Ser	зст Тйг	oce Ala 011	atc Ile	osp qsA	atc Ile	tss nsA	888 Lys 435	gγλ ddf	ass Asn
7596	cdc	дух Тух	att 11e 430	er <i>n</i> dsd	atc Ile	ase neA	aac Asa	∜S2 CJÀ ddc	oop sfA	tss nsA	att 511e	oaa neA	450 Asj dec	зсс	ggn dss	tsp qsA
1248	arg Met	aag Lys 415	сту дая	gee Lys	ger	oza Sccd	410 Ser tcd	gtt Val	tss nsA	ejy ddc	agc Ser	atc Ile 405	drc NgJ	saa 2VJ	gγλ ddc	ger rcd
7500							tcc									
zsīī							dst Asp									
FOTT	atc Ile	asc Asn	gŢλ ddc	365 GJ <i>u</i> 365	dsf.	dst Asp	ggg Lys	agt Ser	д£а Уа1 360	act Thr	gcg Ala	эс <i>с</i> Тит	дрк ЗСЭ	322 GJJ ddf	ааа Гуѕ	gγλ dd¢
9501							аса Тћг									
1008							taa naA 0££									
096	top alA 320	sag Lys	aac Asn	gta Val	gca Ala	tap qeA 315	att Sll	gtg Val	gaa GIu	ааа Гуѕ	sca Ala 310	дрг Трг	Ag J Af A	tta Leu	ej aac	302 207 302
216	gγλ āāc	ggn Ggn	gac Asp	аса ТћГ	300 Ser Gct	tct Ser	gj ddf	tss nsA	ejn dsd	562 GJA ddc	гуз Гуз	gsc gsc	ваа Гуз	CJX ddf	асt Тћг 062	gtt Val
† 98	ttg Leu	sag Lys	GJ AAF	gac Asp 385	гуз Гуз	gaa G1u	ваа Гуѕ	att 11e	9tt Val 280	tct	дст Трг	ggg Lys	gcg Ala	575 677 995	atc Ile	гуз гуз
918	grt Val	gaa GJn	570 Трт 210	ggg Lys	aag Lys	ejl ddc	aac Asn	Sec Yab Gec	aaa Lys	261 9dc	gyn dss	Agg Agg	aat Asn 09S	gtt Val	act Thr	цуц scd
89 <i>L</i>	аса Тћг	999 Pys 255	gcd Thr	tsp qsA	ууş	ger	720 76 <i>n</i> 760	phe ttc	dag G1 <i>u</i>	gtc Val	у Трг	gac Asp 24S	tac Tyr	зст Трг	yrd cdc	Agg acc
720	S40 Fre	dst dsA	gtt Val	aac Asn	dst Asp	235 Ser 26c	gct Ala	дук Зсв	вов Трг	gŢλ āāŗ	530 520 520	ваа Гуѕ	gtt Val	ejl ddc	r X z	316 116 225
Z <i>L</i> 9	ggc Yan	tgg Trp	ду Дас	gct	sac Asn 220	tta Leu	gta Val	gac Asp	ggg Lys	312 781 945	agc Ser	дся	gcg Ala	cdr Gdr	510 F\\ 239	aaa Lys

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	ηeη	rλs	ејп	дуд	740 CJ	Val	zes	Тĥг	пәq	qeA 2££	дуд	гел	qsA	Γλε	130 _F %a	пәq
	zes	Ίλι	дуд	152 БР6	nsA	Thr	суу	neA	150 eju	rys	IJe	Гλг	пөл	nsA 311	qsA	суу
	εlÆ	гуз	IIO ren	тит	əĮI	nŢĐ	Arg	sIA 201	дук	Гел	۲a۷	суу	JOO FÀs	ღუთ	usĄ	ьре
	Lλι	LaV 29	ьſА	qrT	qsA	zəs	nsA 06	еŢп	пŢЭ	Val	rys	82 CJ <i>n</i>	εĶη	сŢл	gŢλ	дуц
	80 CJ	nŢĐ	гуз	qsA	ser	nsA 27	Val	IJe	пәq	ſsV	sIA 07	Val	тут	Ąĸd	ren	LaV 23
	ько	qeA	ren	Туг	nəq nəq	qsA	nŢĐ	n TĐ	ети	22 07 <i>0</i>	пŢЭ	neA	иsĄ	ьlА	26r 26r	БĺА
	етр	Val	дуд	sIA ∂≱	ъре	пәт	Гел	дуц	sIA Op	Γeπ	Val	вſА	Ιμι	72 32	Val	дүд
	БĹĀ	zəg	ьſА 0£	Arg	Γλε	тит	siH	neA 2S	γĸα	дуц	Гел	сγл	26I 26I	Val	Val	1 ₆ V
	dıL	s[A 2[nsA	пәq	ьlА	Ser	neA 01	qıT	IJe	ЭŢI	Arg	nəŋ	IJe		[[<(nzA	
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		dir	065	7.5.	5.00	TDA	720	589	700	n=	[70		089	Γ	6	
9441	វន្តន	tgg Trp	ети	tat Tyr	GJ ^{\(\)} ddf	gtc Val	tot Ser	БĹÁ	tcc Ser	gct Ala	стл аағ	ott Phe	cat His 580	дŢЛ dāc	yrd cdc	Ser
9441	nsA	575 575 617	GJn csg	Ala tat	ddr Lyr	дрс	Lys 570 tct	Ile gca Ala	11e	dcf	Asn	265 GIY	STH	ddc veb	cdc	rcd ITe
	192 062 tss nsA	£dd 212 ddc ddc 325	Tyr cag cag Gln	Gly gct Ala tat	adt acg Thr	Ala ggc Gly Gly	Tyr aaa Lys 570 tot	GLY Stc 11e	Ala att ile	dct tdd Trp	GIY 550 8at Asn ggt	Arg Gly 565 565	Cat His	Thr gac gac	cdc Ser cc	S45 att Ile tcg
8Z <i>L</i> T	Gly agt Ser 560 agt Asn	raa 212 dac dac rcc ser	Ala tac Tyr tcc Ser cag Gln	rst dcc dcc ddc	acd Thr acd Thr Act	dfc ddc yjs dcc ycc	Lys tac Tyr aaa Lys 570 tct	GJY gca gca gca gca gca gca gca	Pro gcc Ala gcc Ile	dcf fdd fdd dgg GJn fen 232	ddc egy ggt ddc ddc	ffc 292 303 303 cdc YI3	Tyr ggc Gly cat His	dac dac dac Thr	cdc ger cdc ddc pen 230	176 272 272 342 330 330
1680	Ala ggc Gly agt Ser 560 aat Asn	tgg ely ely elt	Ala gcg Ala Tyr tcc Ser cag	11e S25 G1y G2y G2y G2y G2y	Ala acg acg atc atc atc	drc GTN ddc GTN dcc GTN ger	Ala aag Lys Tyr aaa Lys 570	300 ACS ACS ACC ACC ACC ACC ACC ACC ACC ACC	tcc gcc gcc gcc gcc gcc gcc gcc	Ala Celu Celu Celu Celu Celu Celu	Arg Gly Gat Gat Aga Gly Gat Aga Aga Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly	FFC 295 PFS GG PFS GGG PFS GGG PFS GGG GFS GGG	tat Tyr ggc Gly ggc Gly	agc gac gac gac gac gac gac gac gac gac	cdc cfd ren cfd	116 617 617 636 617 637
1632	Asn Age Age Age Age Age Age Age Age Age Age	ecc GTA SAC Ser Ser Thr	CFU CSA CSA CSA CSA WIS ACA ACS ACS CSI DACS ACS ACS ACS ACS ACS ACS ACS ACS ACS	Arg GGLY Age GGLY Age GGLY Age GGLY Age GGLY Age Age GGLY Age Age Age Age Age Age Age Age Age Age	dcd Wet 240 afc 340 afc 340 yrs	dec dac dac dac dac dac ger sar css	tet The The The Age Age Age The	Acs Sic	tcc Pro get for GLn get for GLy S20 ggc GLy	dcf cfn cfd cfn cfd cfn cfd cfn cfd cfn cfd cfn cfd cfn cfd cfn cfd cff cfn cff cff cff cff cff cff cff cff	CGT AST LAKE GGTA AST AST	ffc 202 202 202 202 202 202 202 202 202 20	cag Gln Tyr ggc Gly cat His	ggc CLy Asp ggc GLy Asp ggc GLy	cdc grad ddc grad cfg yzb dgc grad dgc grad grab	£Cd 115€ 942 242 242 242 242 242 242 242 242
87LT 089T 7E9T	yeu ser S	redd 2212 2012 2012 2012 2012 2012 2012 20	Thr. Thr.	tat get get get get get get get get get ge	Asp	GTV GGTV GGTV GGTV GGTV GGTV GGTV GGTV	Ltys S70 test S70 tes	Acs STC GTA Adc STC GTA Adc STC GTA Adc STC FAx STC FA	caa gec GLY GCLY GCLY GCLY GCLY GCLY GCLY GCC GCLY GCC GCC GCC GCC GCC GCC GCC GCC GCC GC	dcc Lxb Lxb dgg dgg ren ccd dcd dcd	age Assa Assa Assa Assa Assa Assa Assa Ass	### 265	cst GLY	Adc yeb agc Lyr Agr Agr Agr Agr Agr Agr Agr CC1 Agr Cc1 Yes	cac Ser Condition of the Condition of th	гса чгг стл аас стл аас асу асу

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96 F 06ħ Ile Thr Asn Val Ala Pro Gly Val Lys Glu Gly Asp Val Thr Asn Val 040 Cly Asp Ala Leu Asn Val Gly Ser Lys Lys Asp Asn Lys Pro Val Arg Ser Val Ser Leu Gly Ala Gly Ala Asp Ala Pro Thr Leu Ser Val Asp 440 Asn Gly Lys Asn 11e Asp 11e Ala Thr Ser Met Thr Pro Gln Phe Ser 452 Asp Glu Thr Val Asn Ile Asn Ala Gly Asn Asn Ile Glu Ile Thr Arg Ser Gly Lys Val 11e Ser Gly Asn Val Ser Pro Ser Lys Gly Lys Met Ten Glu Asn Ser Gly Trp Asn Leu Asp Ser Lys Ala Val Ala Gly Ser Thr Val Met Tyr Asp Val Asn Val Gly Asp Ala Leu Asn Val Asn Gln Gly Lys Gly Thr Thr Ala Thr Val Ser Lys Asp Asp Gln Gly Asn Ile 345 ysb rhs bhe Glu Thr Val Thr Ser Gly Thr Ash Thr Phe Ala Ser 330 GIY TEP AIG MET LYS THE THE ALA ASR GLY GIR THE GLY GIR ALA 310 Glu Gly Leu Val Thr Ala Lys Glu Val Ile Asp Ala Val Asn Lys Ala 562 Val Thr Gly Lys Asp Lys Gly Glu Asn Gly Ser Ser Thr Asp Glu Gly rice Ile Gly Ala Lir Ser Val Ile Lys Glu Lys Asp Gly Lys Leu Lyr Tyr Val Aal Glu Ser Lys Asp Asn Gly Lys Tys Glu Val 520 Ast Arg Thr Tyr Asp Thr Val Glu Phe Leu Ser Ala Asp Thr Lys Thr 530 235 ITG TAS CTA AST TAS DIO CTA IDI LATA SER ASP ASD ASI ASP PAC 220 SIZ Lys Lys Arg Ala Ala Ser Val Lys Asp Val Leu Asn Ala Gly Trp Asn 200 Thr Gly Ala Thr Asn Val Thr Asn Asp Asn Val Thr Asp Asp Glu **T82** Val His Leu Asn Gly Ile Gly Ser Thr Leu Thr Asp Thr Leu Leu Asn GIY Leu Asn Phe Ala Lys Glu Thr Ala Gly Thr Asn Gly Asp Thr Thr SST Ser Phe Ser Ala Asn Cly Asn Lys Val Asn Ile Thr Ser Asp Thr Lys

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432	agt Ser	дук ЗСС	ren cfd	зьр qsA	140 140 3cs	ctc	osp qeA	гуз	aaa Lys	132 ren c¢d	tec	tac Tyr	дук чсс	phe ttc	730 261 3dc	26r agt
384	dsc Yab	tss nzA	зсс Тух	aac Asn 225	ggn dgg	tss nsA	зсс Трк	ggc Yan	150 GJ <i>n</i> daa	tss nsA	зсс	aac Asn	css css	ggg Tys	atc IJe	ваа Lys
355	ren crd	aac Asn	JJO Ysb	gjλ ddc	oop siA	ааа Гуѕ	ctc Leu	102 Дук Дук	atc 11e	ују Асч	дуу Дду	gcc Ala	100 r\x sss	cta Leu	gra	aga Arg
288	гуз Гуз	96 egn død	ysp gsc	Буе сгс	tat Tyr	gta Val	аса Б1А 06	LTP Tgg	taa neA	tca Ser	jap gaA	82 67 <i>n</i> 89	урт Трг	GJ \\ ddf	egn das	aaa Lys
240	989 67 <i>n</i> 80	gyy ggs	тук Тук	ejy ddc	ет <i>п</i>	232 Lys 75	jap qsA	Ser	cdr yrd	Бре ссс	JO Ser	nəq ffd	ard Val	grc Val	gcr Ala	301 Thr 30
767	Arg	сва	Val	L LO	09 09	ren	Tyr	пәт	qsA	qeA 23	qsA	qsA	лит	БſА	neA 0∂	БſА
ÞÞΙ	сŢu	gtt Val	лит	sIA др	Бре	гел	гел	туг	sIA 01	пәл	Val	ьſА	тит	ьIA дЕ	Val	дуд
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96	620	554														
96 8 F	qıT	gcc Ala	nsA	ren	ьlА	Ser	nsA 01	Trp	ЭŢІ	IJe	Arg	ΤΥΥ 5	IJе	гуs	nsA	atg
	qıT	sIA SI	nsA	ren	ьlА	Ser	nsA 01	Trp	ЭŢІ	IJe	Arg	tac Tyr 5	IJе	(1 2 333 2 2 2 3 3	ysu 990 5> (1	<222>
	qıT	sIA SI	nsA	ren	ьlА	Ser	nsA 01	Trp	att 11e	atc 11e	Arg	2 Tyr 13 13 13 13 13 13 13 13 13 13 13 13 13	1971) Sta	797 Sesis 50 50 668 868 Lys	yau 990 3> 15 1> (1 1> CI	<pre></pre>
	qıT	JS ACC	590 Tas	ctc	дсc	agt Ser	aat Asan 10	785 785	att 11e	tdis stc	yrd cdc udif	meni (1)	580 (1797)	797 AV Sissis CO (1(1	yan 990 95 (1 75 (1 95 Ne 15 IV 15 IV	######################################
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	LIP LGG Agu	gar gar gcc gcc	Ser 590 Ash	AlaTyr	Thr	Ser Sqt Cly	Lys 570 Ser Asn 10	Flags Arab	Ile Ser	qrT alA eibi:	GJY Parn Parn Parn Parn Parn Parn Parn Parn	G1y S65 Tyr Tyr Tyr	VLS His 580 Strie Firs 119	qsA VLD 197 797 797 797 798 868 968 10(1	yzu yzu 50 (10 10 10 10 10 10 10 10 10 10 10 10 10 1	Wer
	Ser Asn Trp	STZ ATZ	Tyr Ser Gln 590	Fla Alax	дсс УЛЯ ДРЕ ДРЕ ДРЕ ДРЕ ДРЕ 240	Ala SSS Cly Val	Tyr Lys 570 Ser Asn 10	GLY Trp	Ala Ser stt	ulə qrT Ala Ala	G1y Asn G1y Asn Asn	Arg GLY 565 Phe Tyr 707 Arg 708 Arg Arg 708 Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg	TYT GLY YES	Thr Asp 61y 93	yeu yeu 99c (10) 152 (10) 153	% Per ()
	Ser Seo Seo Asn Asn Trp	Ser Arb	Ala Tyr Ser Gln 590	Met GLy Ala Tyr	Acc Ars Lyr Lyr 240	Ser A CLY Vel	Lys Lys 570 Ser Set Asn 10	Tre PTS 2882 CT% CT%	Pro Ser	Leu 535 ulo Trp Ala sibi:	Tyr 61y Asn 61y Ash	Ala Arg Caly 565 Phe Tach 7	Tyr His 580 Stà Ilè	TAR STREET TAR STREET TAR TAR TAR TAR TAR TAR TAR TAR TAR TA	yen	% Per 400

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Substitute Sheet (Rule 26) RO/AU

7500	400 Ser tcc	Jap qsA	req retd	tss nsA	tgg Trp	362 GJA ddr	26r 9dc	ase neA	csa csa	ren cfd	390 GJ u C9d	tss neA	dfc dfc	asc Asn	cta Leu	oce Ala 285
7725	jap qsA	gγλ aac	λg ζ	двв пеА	95a Val 380	tsp qsA	tat IYr	rls ssd	Λ ^g J δες	act Thr 375	atc Ile	aac Asn	gγλ ddc	csa Caa	jap qaA 075	jsp qsA
\$0TT				асt Тћг 365												
9501				аса Тћг												
1008				аса Тћг												
096				ggg Lys												
815				gac Asp												
† 98				285 Ser 285							-	-				
918				ger												
89 <i>L</i>				Agr Afc												
027				CT7 ddf			-									
Z <i>L</i> 9				gtt Val												
6 24				gra Val 205												
915	_		_	GJX ddf						-	-					
828	ст ^х ааа	gct Ala 275	дук чсд	ggg Qgg	гуs	gcg 81A	110 bye fff	aat neA	nəq tçd	ejl ddc	ваа Гуѕ	асс Тћг 165	oap qaA	agc Ser	аса Тћг	atc
081	aac Asn 160	λ ^g ζ	ваа Гуѕ	tes neA	gjλ ddr	aac Asn 251	gca Ala	ejl aac	eya aaa	Ser rcd	tta Leu 120	ggg FÅ2	gaa G1u	act Thr	д ч в СТ <i>п</i>	gtt Val 145

Substitute Sheet (Rule 26) RO/AU

	εſÆ	zes	sIA 0E	yxd	Γλε	тит	siH	nsA 2S	Arg	дуц	ren	сŢπ	26r 26r	Val	Λgl	Val
	Trp	sIA 21	пеА	гел	ьſА	ser	nsA 01	qıT	IJG	IJG	γxd	Tyr 5	ΙŢĠ		usų U> T	
									\$	sibi:	tipa.	inəm	экта	86 T/	9N <8 5> bi 1> 20 10	<515 <515
<i>L</i> 6 <i>L</i> T										taa					dfc Agg	
9 <i>LL</i> T	gcs Ala	Ser	јор Б1А 062	gyy ggt	ьре ггс	cat	gŢħ āāc	cdc Frd 585	zez	taa neA	gŢλ ādc	tcc	gct Ala 082	Thr acg	стл ddc	sag Lys
82 <i>L</i> I	atc Ile	gtt Val 878	Ldd rdd	taa Ash	ет ^д ааа	зсt Трк	gac Asp 670	tot Ser	att Ile	26I 9dc	2er £cd	tac Tyr 365	eyy aac	atc 11e	gcc Ala	tac Tyr
1680	260 GJY ddf	gcc 818	gaa G1u	gγλ ddc	yxd cdc	tat Tyr 355	аст Тћг	eyl adr	eγλ ààc	ej ddc	9£0 176 220	y J 9 aca	atg Met	atg Met	agt Ser	242 PÀs ssà
7632	e7ì dac	ccc	гед Гер	tat Tyr	gcg Ala 042	ду Суд	gct Ala	ren rtd	ejl adf	sop slA 282	эсс Тит	sp gca	att 511e	gcg &LA	230 CJu css	oop 81A
728¢	atc Ile	GJ Å ddf	gcg Ala	cdc SS2	gcg Ala	aac Ash	gj ddc	gac gañ	250 A97 A£A	tss nsA	gac Asp	stc Ile	yrd cdc	aac Asn 215	ggc Yan	ren ttd
1236	aac Ash	csa Gln	gcg Ala 012	gtg Val	eyl ddf	ааа Lys	ctt	202 Gyu css	gca Ala	dfc Agj	узу Узу	дук Тук	965 Val 500	tsp qsA	с _Т л ааа	deg Gjn
1488	888 Lys	gtt Val	ej aac	bro ccd	gcc Ala	Λ ^g Ţ	taa naA 064	дук Дук	att Ile	√ √zā cāc	afc Afc	ccc Pro 485	ggg	aac Asn	gcc Ala	gat Asp
1440	480 Lys	ger	GJ \\ ddc	gtc Val	tee neA	trd ren	ууэ дсд	дŢЛ dāc	deg dag	ysb dsc	jap qaA 0⊺≱	grg	26r 9dc	tta Leu	got.	465 Pro CCC
1392	gcg	jap qaA	gcg Bla	стл ааа	gcg 61A 064	gjλ ddc	ctc	26r £cd	λg ζέτ	422 261 9dc	2er Ser	ъре ггг	су и су у	bro ccd	420 11 420	atg Met
7344	ger rcd	дрк Трк	gcc alA	atc 11e	gac qsA	atc 11e	aat Asn	ggg	440 CJλ άδc	aac Asn	cdc yrd	дух эсс	att 11e	432 C7 <i>n</i> ded	atc Ile	aac Asn
1596	aac Asn	ejl ddc	gcc Ala 430	aat Asn	att Ile	ysu ggc	dfc dfc	acc Thr 3Sp	дуя дуу	dst Asp	atg Met	ggd Lys	∜50 Суλ аав	sag Lys	ger	bro ccd
1248	2er rcd	415 Val 9tt	tss nsA	стл ddc	ger	atc	410 Agi	aaa Lys	дŢЛ āдс	2er rcd	tct	402 CJλ dd⊊	gca Ala	gtt Val	gcg Ala	гуs

τΛΧΧ

7E115/66 OM

PCT/AU98/01031

Substitute Sheet (Rule 26) RO/AU

380 375 yab yab gru gra yau ije iyi nat rak yab nai yau nai cia yab 9€ rys Val Thr Phe Ala Ser Gly Asn Gly Thr Thr Ala Thr Val Ser Lys 342 Gly Gln Thr Gly Gln Ala Asp Lys Phe Glu Thr Val Thr Ser Gly Thr 330 Asp Ala Val Asn Lys Ala Gly Trp Arg Met Lys Thr Thr Ala Asn STE OTE Ser Ser Thr Asp Glu Gly Glu Gly Leu Val Thr Ala Lys Glu Val Ile 562 Grn ras wab Gly Lys Leu Val Thr Gly Lys Gly Lys Asp Glu Asn Gly 280 Gly Lys Lys Thr Glu Val Lys 11e Gly Ala Lys Thr Ser Val 11e Lys Ser Ala Asp Thr Lys Thr Thr Thr Val Ash Val Glu Ser Lys Asp Asn Ser Asp Asn Val Asp Phe Val Arg Thr Tyr Asp Thr Val Glu Phe Leu Leu Asn Ala Gly Trp Asn Ile Lys Gly Val Lys Pro Gly Thr Ala Yau Asi Thr Asp Asp Glu Lys Lys Ard Ala Ser Val Lys Asp Val 200 Thr Asp Thr Leu Leu Asn Thr Gly Ala Thr Asn Val Thr Asn Asp Thr Asn Gly Asp Pro Thr Val His Leu Asn Gly 11e Gly Ser Thr Leu OLI IJG IDI SGL WSD IDI TWS GJW TGD WSG BJG TWS GJW IDI BJG GJW Ast Giu Thr Giu Lyr Leu Ser Phe Giy Ala Asn Ciy Asn Lys Val Ash 332 Set Set bye Thr Tyr Ser Leu Lys Asp Leu Thr Asp Leu Thr Ser 150 Lys Ile Lys Gln Asn Thr Asn Glu Asn Thr Asn Clu Asn Thr Asn Asp SOT Arg Val Leu Lys Ala Gly Ala Ile Thr Leu Lys Ala Gly Asp Asn Leu 06 ras ciu ciy Thr Giu Asp Ser Asn Trp Ala Val Tyr Phe Asp Giu Lys Thr Ala Val Val Leu Ser Phe Arg Ser Asp Lys Glu Gly Thr Gly Glu yfs yzu yfs Lyr ysb ysb ysb ren Lyr Leu Glu Pro Val Gln Arg Thr Val Ala Thr Ala Val Leu Ala Thr Leu Leu Phe Ala Thr Val Gln

XXATT

Substitute Sheet (Rule 26) RO\AU

ÞÞI	eju csd	gtt Val	acg Thr	gca Ala 24	Phe ttt	ttg Leu	ren cçd	дук зсд	gcg Ala 10	ren ttd	gra Val	gcc Ala	эсс Тук	899 192 32	gtg Val	дух ЗСС
96	sca s1A	tcc	oop sla 30	yzd cdc	ваа Гуѕ	дух зсс	cac His	aac Asn 2S	odc cdc	вса Тћг	ctc	n Ţŋ ded	tcc \$er	gta Val	gcc Ala	gtc Val
81	tgg Trp	oce Ala 21	tas Ash	ctc	gcc Ala	sgt	tss neA 01	tag Txp	att 11e	atc Ile	cgc Std	cac Tyr c	ata Ile	999	89C 99C 98U	atg
												((7800	S([> CE	
									\$	sţpţ:	ţţbu	inəm	8 Ļ J ē	008	3> NE 3> DN 1> 16	<515 <511
											Trp	ети	Ţλr	295 GJ	Val	zes
	ьſА	zəs	ь1А 062	ету	ьре	siH	сту	p1A 282	zes	nsA	етх	zer	61A 082	дуц	суγ	гλз
	all	LaV 275	Trp	пеA	етл	тит	qsA 072	Ser	IJG	zes	zəs	17r 565	еуλ	IJG	slA	Τγε
	260 540	ьlА	ејл	суу	Уĸа	1yr 555	тһт	суу	еул	етл	220 IJ¢	sIA	Met	Met	ser	242 742
	сту	Бко	пәŢ	Tyr	Ala 0≱2	сŢи	sſĄ	Гел	стх	sIA 2£2	дуц	sIA	ЭŢІ	ьſА	230 CJu	ьſА
	IJe	еух	БĺА	Prg 525	εſĄ	nsA	етл	qsA	787 520	nsA	qsA	IJG	Αχα	neA 212	nsA	пәт
	nsA	сул	sIA 013	Val	суу	Гуs	пәq	202 CJU	ьſА	ſsV	nsA	Thr	7a1 500	qsA	сту	ејп
	Γλε	16V 295	еул	ько	ьſА	ſsV	nsA 0eÞ	тит	IJG	Arg	Val	Pro 485	rγs	neA	slA	qsA
	780 780	zes	етл	Val	nsA	475 Leu	БĺĀ	еул	ејп	qsA	qeA 074	Val	zec	nəŢ	тит	465 465
	БÍĀ	qsĀ	БĹĀ	суу	81A 09p	сту	пəп	zes	Val	422 261	ser	ьре	ети	Pro	19 120	Met
	Ser	дψД	вÍА	911 116	qsA	IJe	паА	Гуs	440 €7λ	иsĄ	Arg	Thr	ΙJG	432 CJn	IJG	nsA
	nsA	сту	sIA 0£≱	nsA	IJG	nsA	Val	425 Thr	ејп	qsA	Met	гүs	₹50 CJλ	rλs	zes	Βτο
	Ser	Val 415	nsA	еуу	zec	IJe	18V 410	εγΊ	сту	zes	zes	₫02 ሮፓλ	slA	LaV	slA	гλз
	400 261	qsÁ	Гел	пеA	Trp	395 GJA	zes	nsA	стр	Гел	390 CJ ^u	nsA	Val	nsA	ren	ь1А 28£

Substitute Sheet UA\OA (82 e)

216	tss nsA	egn dad	gγλ ddc	ааа Гуѕ	300 GJ \\ ddc	aaa Lys	gjλ ddf	дук Дук	gtt Val	792 190 190	sag Lys	GJ \\ ddf	gac gac	гуs	399 039 039	ggg Lys
†98		gtt Val														
918		гуs														
89 <i>L</i>		522 GJ <i>n</i> ded														
02 <i>L</i>		у Тћг Трг														
Z <i>L</i> 9																dsc dsc
624		Дук эсс				•			•	_	_	_		_		ttg ren
978		2er Ser														
828		112 112 112														
085		ааа Гуѕ														
432																gac Asp
384																ren crd
336																cgc Kja
882																ggn dgg
240	80 GJ \\	taa Asn	gγλ ààc	дуя дуу	ggg Lys	Jap qaA 27	atc Ile	atg Met	ъре стс	csa csa	10 ren	ard Ard	ren ctd	gct Ala	zer rct	cdc Frd 92
761	gta Val	gta Val	bro ccc	dsa dsa	ьтт Беи 60	ejn dsd	ggn das	gyn daa	ду дву	тър qeA де	gyn Gyn	зьр qsA	эсс Трт	gct Ala	заг ЛаА 02	gcg Ala

7E11E/66 OM

xxx

1728	att	гда	aat	dds	aac	дяс	tcc	att	agt	tcc	tac	aac	atc	acc	tac	άđρ
089τ	gcc Ala 092	dsa G1u	ej dac	odc cdc	tat Tyr	act Thr 355	gγλ ddc	ду дас	ejy adc	atc 11e	gcg A1a 022	atg Met	atg Met	agt Ser	ggd F	242 CJ \\ ddc
1632	ccc Pro	ren crd	tat Tyr	gcg Bla	240 CJu csd	gtt Val	crd crd	gŢX dd£	gca Ala	232 Туц ЗСС	gcs Ala	att 11e	gcg 818	CJ v C99	gcc Ala 530	atc Ile
728đ	ejl ddc	gcg gcg	tgo Prg	pop slA 2S2	aac Asn	GJ Å	gac AsA	ara Ara	taa neA 0S3	gac gac	atc Ile	yrd cdc	aac Asn	aac Asn 515	ren ttd	yar yar
1236	сув С	gcg Ala	210 Agj açà	gyl ddc	ааа Lys	ctt	сва Сдр	sop slA 202	arc Agr	aac Asn	дук всв	gtt Val	tsp qsA 002	с у у дад	n T 9 ded	aaa Lys
1488	gtt Val	∜95 GJÀ dàc	5ro ccd	occ 818	drc Agj	jss nsA	ч 100 Трк ч 100	att Ile	cdc	λg ζες	ccc	482 Lys 485	aac Asn	oce ala	tap qeA	aag Lys
0661	480 261 9dc	gγλ ddc	gtc Val	tss nsA	ren ttd	gcg Ala ara	gγλ ddc	aag Lys	gac Asp	tsp qsA	drd Λg∫ đlo	ser sgc	ren ttd	дук Тук	bro ccc	gcg Ala 465
7385	gat Asp	gcg Ala	стл ааа	gcg Ala	400 G√λ āāc	ctc	2er £cd	λal Ωετ	ser	422 Ser tcc	२५४ २२२	eyn csd	bro ccd	усс Трх	420 Wet	tcg
1344	дук Тук	gcc Ala	atc Ile	gac Asp 245	atc 11e	taa Asa	ааа Гуѕ	gj ddf	aac Asn 440	yrd cdc	дук эсс	att Ile	eyn dag	atc 11e 435	aac Asn	gsc ysu
1296	ej ddc	occ alA	tss neA 0£p	att 11e	aac Ash	gtc Val	дух ЗСС	939 61 <i>u</i> 63 <i>b</i>	dat qsA	atg Met	rys rys	дŢλ āds	450 Fys	26r 9dc	bro ccd	ger Ser
1248	gtt Val	taa naA 214	GJ Y ddc	sgc	atc Ile	Λ ^g J	410 Fàs	ejl ddc	ger	ger Ser	суλ ddf	gcs Ala 201	gtt Val	pop Ala	saa Lys	2er 2er
1500	Jap qaA 001	red Leu	tsa nsA	Lrb rad	сту ddf	362 261 9dc	ggc ygu	css css	ren crd	csg	jaa Asn 390	Ag J Af C	aac Asn	cta Leu	oop s14	gat Asp 385
7725	gjλ ddc	gtc Val	tss nsA	gta Val	gat QsA 08E	tat Tyr	r ys	gtt Val	gor Thr	312 116 312	ggc Yan	ejl adc	css css	jep qsA	dat geA 07E	aaa Lys
770¢	Ser	gta Val	асt Тhr	gcg 818 365	дрк Дрк	дук Дук	стл ddf	ggg	300 GJÀ ddç	sgt Ser	gct Ala	ъµ⊖ rrr	дук Тук	g£a Val 355	sat Asn	дук Ч
9501	gyl ddc	tca Ser	320 Thr 350	A97 Aff	дук чсс	gaa Gjn	ъуe ггг	942 Pys 345	gac Asp	gct Ala	C99	ст ^у аағ	аса Тћг 340	сяя сяя	сул aaf	ast neA
1008	gct Ala	332 Thr 36c	дук Тук	дух чсэ	eee Lys	atg Met	aga Arg 330	tag Trp	ст ^х аағ	gct Ala	rys	aac Asn 325	gta Val	gce Ala	jap qaA	att Ile
096	350 Agj d£d	eyn dss	aaa Lys	gca Ala	цук цук	312 A91	tta Leu	дŢЛ ddc	gyn Gyn	дŢЛ ddc	310 GJ <i>n</i>	gac gac	вса Тћг	tct Ser	tct Ser	302 CJÀ đđ¢

Substitute Sheet (Rule 26) RO/AU

520

Ala Ser Asp Asn Val Asp Phe Val His Thr Tyr Asp Thr Val Glu Phe Ast Leu Asn Ala Gly Trp Asn Ile Lys Gly Val Lys Pro Gly Thr Thr Asp Ash Val Thr Asp Asp Lys Lys Arg Ala Ala Ser Val Lys Asp 210 210 ren Lyr yab Lyr ren ren yau Lyr Gjå yjs Lyr yau Asj Lyr yau **58T** Gly Thr Asn Gly Asp Thr Thr Val His Leu Asn Gly Ile Gly Ser Thr OLT Yau 11e Ibr Ser Asp Thr Lys Gly Leu Asn Phe Ala Lys Glu Thr Ala SSI Ser Val Glu Thr Glu Lys Leu Ser Phe Gly Ala Asn Gly Asn Lys Val **321** Asp Ser Ser Phe Thr Tyr Ser Leu Lys Lys Asp Leu Thr Asp Leu Thr ISO ren rise Ile Lys Gln Asn Thr Asn Lir Asn Glu Asn Thr Asn His Wan Thr Leu His Gly Ala Thr Val Thr Leu Lys Ala Gly Asp Asn ejn yau ejn set lyt ejh yau ije ejh ltb set ije lht lht yab yau Arg Ser Ala Leu Val Leu Gin Phe Met ile Asp Lys Glu Gly Asn Gly YIS ASD ALE Thr Asp Glu Asp Glu Glu Glu Leu Glu Pro Val Val Thr Val Lys Thr Ala Val Leu Ala Thr Leu Leu Phe Ala Thr Val Gln 97 Val Ala Val Ser Glu Leu Thr Arg Asn His Thr Lys Arg Ala Ser Ala OΤ Wet yeu lys lle Tyr Arg lle Ile Trp Asn Ser Ala Leu Asn Ala Trp <213> Neisseria meningitidis <212> PRT <5117> 266 <510> 12 Ala Ser Val Gly Tyr Gln Trp gca cer geo 55-वेटड एटए वेएट वेवेट एडर टडवे एवेवे एडड 065 585 IJG FNS CJN LDI YJY SGI CJN YSU SGI YIG CJN HIS BUG CJN YJY SGI sec ses dae sed der rec dae ser red ede dar er fre dar der fee

TXXX

Gly Tyr Ala 11e Gly Tyr Ser Ser 11e Ser Asp Gly Gly Asn Trp 11e

049

1800

UA\OA (8s sluA) Substitute Sheet

Ala Ser Val Gly Tyr Gln Trp 282 Ile Lys Gly Thr Ala Ser Gly Asn Ser Arg Gly His Phe Gly Ala Ser 049 Gly Tyr Ala 11e Gly Tyr Ser Ser 11e Ser Asp Gly Gly Asn Trp 11e 099 Gly Lys Ser Met Met Ala 11e Gly Gly Gly Thr Tyr Arg Gly Glu Ala 232 lie Ala Gin Ala Ile Ala Thr Ala Gly Leu Val Gin Ala Tyr Leu Pro 250 Asn Leu Asn Asn Arg 11e Asp Asn Val Asp Gly Asn Ala Arg Ala Gly Lys Glu Gly Asp Val Thr Asn Val Ala Gin Leu Lys Gly Val Ala Gin Lys Asp Ala Asn Lys Pro Val Arg Ile Thr Asn Val Ala Pro Gly Val 0 L 🗗 Ala Pro Thr Leu Ser Val Asp Asp Lys Gly Ala Leu Asn Val Gly Ser Ser Met Thr Pro Gln Phe Ser Ser Val Ser Leu Gly Ala Gly Ala Asp

Ser Lys Ala Val Ala Gly Ser Ser Gly Lys Val 11e Ser Gly Asn Val Asp Ala Leu Asn Val Asn Gln Leu Gln Asn Ser Gly Trp Asn Leu Asp Lys Asp Asp Gln Gly Asn 11e Thr Val Lys Tyr Asp Val Asn Val Gly 360 Thr Asn Val Thr Phe Ala Ser Gly Lys Gly Thr Thr Ala Thr Val Ser 345 yen cly cln Thr Gly Gln Ala Asp Lys Phe Glu Thr Val Thr Ser Gly

000 Yau yau 11e Clu 11e Thr Arg Asn Cly Lys Asn 11e Asp 11e Ala Thr

455 Ser Pro Ser Lys Gly Lys Met Asp Glu Thr Val Asn Ile Asn Ala Gly

0Tb

330

312 Gly Ser Ser Thr Asp Glu Gly Glu Gly Leu Val Thr Ala Lys Glu Val 562 rks egn rks ysb egk rks ren Ast Thr ely Lys ely Lys ely Elu Asn 280

Ile Asp Ala Val Asn Lys Ala Gly Trp Arg Met Lys Thr Thr Ala

Yau Cly Lys Arg Thr Glu Val Lys Ile Cly Ala Lys Thr Ser Val Ile 597

Leu Ser Ala Asp Thr Lys Thr Thr Val Asn Val Glu Ser Lys Asp

Substitute Sheet (Rule 26)

6 29			dst gsA													
9 <i>L</i> \$	tas neA	crd	T 60 ren	atg JeM	Jap qaA	дук ЗСС	ren	30¢ 19 182	ger £cd	ejl age	stc stc	ejl adr	180 Yau 081	cfg cfg	cat	gtt Val
228			gac Asp													
081			gac Asp													
432			gaa G1u													
384			у Трт													
988			IIO ren crd													
288			gaa G1u													
240			26r 9dc													
767			tcc													
ታ ታ ፒ	_		цуг чсд						_	_	_	-			-	
96			oop ala 08													
85			tss nsA											999		atg
												(1	5LLT) • • ([) <: > CL	2 22>
									\$	sipi:	ı, bu	i nəm	вíта	6 L		<513 <515 <511

565

Substitute Sheet UA/OA (82 sluA)

7440	дŗс	၁၁၁	999	990	асс	gat	aag	эдс	ddc	drc	385	εεd	dcd	ddc	ded	дяс
1392	jap qaA	ara ara	ger	tta Leu	зов ТАТ 09р	ccc	gcg Ala	jap qsA	y j s dcd	422 CJ AAA	gcg Ala	ej Adc	ctc Leu	26r £cd	955 Val 950	261 9dc
1344	ger Ser	Бре ССС	суя Суя	442 bro ccd	дук Тук	atg Met	ger	gct Thr	gcc 440	atc Ile	gac Asp	atc 11e	taa neA	432 Lys 435	gŢλ āāc	aac Asn
1596	cac Yrd	дук чсс	att Ile 430	ern dsd	atc Ile	sac Asn	aac Ash	∜52 CJλ ddc	gcc Ala	tss nsA	att Ile	sac Asn	450 Ag⊺ d£c	урт Трх	gaa G1u	dat qaA
1248	atg Met	412 Pys aag	ggy dds	аад Гуѕ	ser	bro ccd	410 Ser £cd	gtt Val	taa naA	gγλ aac	ger sgc	atc 11e 405	∆g ∂¢c	гуs	gγλ ddc	ger tcd
1500	tot Ser 400	gj) ddf	gcs Ala	Arr Arr	gcg Ala	888 Lys 395	tec	jap qsA	red red	tss nsA	tag Trp 390	ejl daf	26r 9dc	aac Asn	csa csa	382 ren 382
1125	су u csd	taa naA	gtc gtc	aac Asn	сғя Төл 380	gcc gcc	jsp qsA	gγλ ddc	arc Agr	taa naA 27£	gta Val	jap qaA	tat Tyr	rys aag	310 791 310	дук зсғ
POTT	atc 11e	aac Asn	gj ddc	365 GJn 362	dst gsA	jap qeA	sss Lys	sgt	д£а Уа1 360	дуг Туг	gcg acd	аст Тћг	аса Тћг	322 GJJ ddf	tss nsA	дуу Дағ
9501			320 bye rrr													
800T			GJ\ ddf													
096	gct Ala 320	ged Lys	aac Asn	gta Val	gca £1A	tap qaA 315	att 11e	Λ ^g Ţ άρλ	gaa G7n	ggg Pys	gcs Ala 310	трт ТфТ	gey Val	tta Leu	GJÀ đđc	302 302 302
216	gŢλ āдс	ggs GJn	yst	аса Трг	ser 300	tct Ser	gj ddf	jas nsA	egn død	582 CJA ddc	ggg	ejl ddc	aaa Lys	GJY GGt	зсt Трг 290	Agr Afr
 ₹98	trg ren	ggg	đας	gac Asp 285	aaa Lys	ges daa	rys Lys	att Ile	280 Val 9tt	tct Ser	дст Трк	sag Lys	gcg 818	512 67 88¢	atc Ile	aaa Lys
918	gtt Val	gga Qga	570 Трт 3СС	aaa Lys	rys	дуу ддс	aac Asn	gac Asp 392	rys aaa	ger	ggg GJn	Λ ^g Ţ ά¢ὰ	taa Asa 03S	gtt Val	зсг Трг	цуц чсд
89 <i>L</i>	дук чсв	522 Lys 233	дуг Тух	jap qaA	gca Ala	261 9dc	S20 ren rrd	ъре стс	egn død	λ ^g J δ¢c	ург Трг	gac Asp 245	tac Tyr	act Thr	cdc	Дgу
720	S40 bye ffc	jsp qsA	gtt Val	ysv ysv	tsp qsA	235 Ser tcc	gct Ala	урк Трх	у Трх Трх	стл ddf	530 5xo 5xo	ggg Lys	gtt gtt	gjl ddc	глг эээ	att Ile 225
2L9	aac Asn	tag Trp	gγλ ddc	дся	sac Asn 220	tta Leu	gta Val	yab dsc	гуs	grt Val 215	ger sgc	дся	gcg Ala	car car	510 Făs	aaa Lys

Substitute Sheet (Rule 26) RO/AU

<510> 17
<511> 592
<513> Meisseria meningitidis

6*LL*I 553 585 Asn Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr Gln Trp sat tog oge gge tte ggt get tee get tat cag tag 9 4 4 1 SLS 049 Ser Ile Ser Ala Gly Gly Asn Trp Ile Ile Lys Gly Thr Ala Ser Gly age att tee gee gge gat tgg att ate aaa gge gee gee gee 821 T Gly Gly Gly Thr Tyr Leu Gly Glu Ala Gly Tyr Ala 11e Gly Tyr Ser dac ade act tat cte age gas gee ggt tat gee ate tae tea 089T 005 232 Ala Gly Leu Val Gln Ala Tyr Leu Pro Gly Lys Ser Met Met Ala Ile I 935 des dar erd arr esd ded rer erd eec dae syd sta srd ded sre 250 Asn Val Asn Gly Asn Ala Arg Ala Gly Ile Ala Gln Ala Ile Ala Thr अबर वेर्त बबर वेतेर बबर वेटवे टवेर वेटवे वेतेर बहर वेटर टबब वेटवे बहर वेटब बटर 788T 909 Val Ala Gln Leu Lys Gly Val Ala Gln Asn Leu Asn Arg Ile Asp वेहट वेटवे टड़ड टह्ह डड़ड वेवेह वेहवे वेटवे टड़ड डड़ट हहते डड़ट टड़ट वड़ट वेडट 1236 061 YEG IJE LYE YEU AST YIS ETO GIY VAL LYE GIU GIY ASP VAL TAE ASR टतेट अर्ट अटट अवर तेट्ट टट्वे तेवेट तेर्ट अडड तेवेवे तेवेवे तेव्ह तेर्ट अटड अअट **1**488 913 04.5 Asp Glu Gly Ala Leu Asn Val Gly Ser Lys Asp Ala Asn Lys Pro Val

Substitute Sheet UA/OA (82 9luA)

480 480	Pro	Γλε	пsА	ьſА	qeA ¿۲4	rys	Ser	етл	Val	nsA 07Þ	ren	БÍA	етл	eŢn	qsA 234
qsA	۸۹J	ser	пәт	400 400	δτο	sſA	qsA	sIA	422 CJJ	sIA	етл	пәղ	zəs	Val 450	Ser
ıəs	ьре	пſЭ	Pro 445	дуц	JəM	ser	тит	sIA Opp	IJe	qsA	IJe	пsА	432 132	сŢХ	nsA
Arg	тут	11e	nŢŋ	IJe	nsA	пеA	452 GJÀ	ьſА	nsA	IJG	neA	450 450	ΙŲΣ	етп	qsA
Met	412 FÀ2	сту	Γλε	zes	Pro	36r 36r	Val	пsА	суу	zəs	₫02 IJG	Val	Γλε	сту	zəs
198 261	сту	ьſА	Val	εſΑ	395 395	ıəs	qsA	nəq	nsA	71p 390	ету	Zer	nsA	eŢu	382 Ten
еޤ	nsA	Val	nsA	380 Ten	ьſА	qsA	еул	Val	nsA 275	Val	qsA	LAL	rys	787 370	дуд
IJe	паĀ	етл	365 395	qsA	qsA	rys	zes	1 ₆ V 360	тит	εſĄ	дуц	тит	322 322	пеА	етл
zəs	εlA	320 БУ	дуц	IsV	гуз	тут	342 GJJ	ıəs	тит	Val	тйт	3¶0	ьре	гуз	qsA
sſΑ	332 CJu	еул	дуц	етр	сγλ	п еА 0££	εlA	дуд	дуц	тит	352 FÀs	Met	Arg	ďχL	етλ
ā ſÆ 0S€	гуз	nsA	Val	εlÆ	qaA 31£	IJe	Val	ејп	гуѕ	alA Ole	дуд	Val	гeл	суу	302 CJ <i>n</i>
СŢХ	етл	qsA	дуд	300 361	zes	сτλ	nsĀ	nŢĐ	562 CJ	гλг	сγλ	гуs	сγλ	191 190	Val
пəq	гЛз	еул	qsA 28S	гλг	сŢπ	Гуs	IJe	787 280	ser	дуц	rys	ьlА	512 CJ X	IJe	ςλη
ĮsV	етп	ТЪг 072	Γλε	rys	суу	nsA	qaA 33S	гуз	ser	етп	[5V	nsA 03S	Val	дук	дуд
тйт	722 722	дуц	qsA	БĺА	Ser	520 Fen	ьре	сŢп	Val	тит	qaA ZAS	Tyr	тит	Ука	ŢŀΛ
540 БУ6	qsA	Val	asA	qsA	232 261	εIA	lhr	дуд	сγλ	530 530	гЛз	Val	сту	гλз	325 IT6
nsA	Trp	еул	ьlА	naA 0SS	nəŢ	Val	qsA	гλз	797 712	Ser	БÍĀ	ьIA	βzĄ	SIO FÀs	гХз
стп	qsA	qsA	Thr	Val	пеA	qsA	nsA	лит Трх	Val	nsA	тут	Thr	61A 2€1	сух	тит
nsA	Гел	I 80 Fen	Met	qsA	Thr	ren	182 182	zes	суу	əĮĮ	етл	nzA 081	рел	siH	Val
тŲТ	Thr	qeA	đŢλ	пеA	тит	710 CJÀ	БĺÁ	дуц	сŢп	гλа	ь IA 23 I	ьре	nsA	Гел	сту
1 e0 r X a	Thr	qsA	Zer	all	122 IJ¢	иsĄ	Val	rys	rλa	120 CJÀ	nsA	БĺĀ	суу	ьре	142 261
ren	Γλε	еуп	дуд	140 C1 <i>n</i>	Val	nsA	IJe	ren	132 CJ	тит	Гел	qsA	εγď	730 730	пәŢ

iivxxx

sec dag etc aca ege eac eaa ege dec tec gea ege ege Ala	
the tac cgc atc att tgg aat agt gcc ctc aat gcc tgg 2 Tyr Arg 11e 11e Trp Asn Ser Ala Leu Asn Ala Trp 5	
(OLL	<222> (1)(1
sia meningitidis	<210> 18 <211> 1770 <212> DWA <213> Weisser
SIV His Phe Gly Ala Ser Ala Ser Val Gly Tyr Gln Trp 580	
918 Gly Gly Asn Trp Ile Ile Lys Gly Thr Ala Ser Gly 585	Ser Ile Ser i
Thr Tyr Leu Gly Glu Ala Gly Tyr Ala 11e Gly Tyr Ser	242 CJA CJA CJA J
Aal Gln Ala Tyr Leu Pro Gly Lys Ser Met Met Ala Ile	Ala Gly Leu 7
SSS SSO SSO SSS SSS SSS SSS SSS SSS SSS	neA lsV neA 818
cen Lys Gly Val Ala Gln Asn Leu Asn Asn Arg Ile Asp	
Ash Val Ala Pro Gly Val Lys Glu Gly Asp Val Thr Ash 485	Arg Ile Thr i
TTAXXX	

96								gtc Val	
85									atg Met I

S Ð ОÞ Thr Val Ala Thr Ala Val Leu Ala Thr Leu Leu Ser Ala Thr Val Gln ББТ sec did ded see dee itd ded ses eid eid iee des sed dir esd

gog aat got acc gat acc gat gaa gat gaa gag tta gaa too gta goa Ala Ala Asn Ala Thr Asp Thr Asp Glu Glu Glu Leu Glu Ser Val Ala **76**5

0LArg Ser Ala Leu Val Leu Gln Phe Met 11e Asp Lys Glu Gly Asn Gly 240 cac for got otg gig the caa the atg ate gat aaa gaa gge aat gga

06 28 Clu ile Glu Ser Thr Gly Asp ile Gly Trp Ser ile Tyr Asp Asp विक करें पुरे करक पुष्ट करक पुष्ट करक पुष्ट पुष्ट करें करक रक्ष कर रेंद्र पुरे पुरे करेंद्र पुष्ट पुष्ट पुष्ट 288

SOT His Asn Thr Leu His Gly Ala Thr Val Thr Leu Lys Ala Gly Asp Asn 336 cec esc ect cte cec ddc dce ecc dft ecc ctc eae dcc ddc dec eac

ISP 150 ren ris 11e ris cju ser cji ris ysb bhe Thr Tyr Ser Leu Lys Lys crd see sic see cas ado ddc see dec rcc rcc crd crd see ₹8£

UA\OA (8s sluA) Substitute Sheet

Substitute Sheet (Rule 26) RO\AU

1500	999	aac	tcg	tot	ddρ	dcs	đεε	dcd	999	tcc	gat	сęд	aat	£ãã	άđρ	эдс
7777	увс Узи	csa caa	ren	eju csd	tsa nzA 08£	Ag J āfc	aac Asn	cta Leu	gcc dcc	jap qaA 375	gjλ aac	∆97 ∂¢c	tss neA	gta Val	jap qaA 075	tat Tyr
FOTT	gag Lys	grt Val	трк трк	atc 11e 365	aac Asn	ejl ddc	csa csa	gat qeA	gat geA 360	ваа Гуз	ser	gta Val	act Thr	gcg Ala 355	act Thr	аса Тћг
302e	ggr ggr	јаа паА	320 GJ \ ddf	sgt	jop síA	₽γ σ ∓∓∓	трк зсс	gta Val 345	гуз Гуз	тук чсэ	дŢЛ ddc	tca Ser	340 Thr 340	gtt Val	зсс	два СТл
8001	БЪе сее	332 172 332	gac gac	jop Ala	суу	ejl ddf	330 Thr 330	сва Сав	дŢЛ ddf	taa naA	jop Ala	355 Thr 325	дуг Тух	вса Тћг	ваа Lys	atg Met
096	aga Arg 320	Lrp raa	gγλ	jop alA	aag Lys	aac Asn 315	gta Val	gca 61A	jap qsA	att 11e	310 Agg 310	gyn dgg	ааа Гуѕ	gcs gcs	дрг Трх	302 Agj afa
216	tta Leu	ejl ddc	dsa Glu	GJ y	300 GJ <i>n</i>	gac Asp	аса Тћг	tct Ser	tot Ser	562 CJA ddf	taa neA	ejn dsd	ejl ddc	ааа Гуѕ	590 GJA ddc	уда Гуз
ħ98	ej aaf	thr Thr	Λ α Ί δεε	ttg Ten	rys aag	сту Дағ	yap dsc	aaa Lys	580 GJ <i>n</i> dss	aaa Lys	att Ile	grt Val	tot Ser	sct Thr 275	aag Lys	gcg Ala
918	ст ^у аағ	atc Ile	888 Lys 270	gtt Val	gaa G1 <i>u</i>	эсс Түт	aga Prd	S e 2 Fys	GJ7 ddc	aac Asn	ysp dsc	aaa Lys	500 261 9dc	gaa Glu	Agg aca	taa naA
89 <i>L</i>	gtt Val	SSS Thr 3ct	цук scd	дух ЗСЗ	saa Lys	дуц Туц	gat Asp 02S	gcs	ger sdc	ren ren	ъре Ере	542 GJ <i>n</i> dsd	grc Val	зся Трг	gac gap	set Tyr
0 <i>ZL</i>	act Thr 240	cac	gtc gtc	БР6 ССС	tsp qsA	332 735 735	tss nzA	два СТл	tca Ser	сва Сав	530 GJ Adf	дрг Трх	Дук эсэ	tca Ser	gγλ dac	асt Тћг 225
7 <i>L</i> 9	sss syl	átt Val	gj y ggt	rys aag	316 116 220	taa neA	rad Lad	gγλ ddf	gcg gcg	tss neA 21S	req req	Ag Afà	jep qeA	aag Lys	310 116 210	agt Ser
624	sop slA	gca 81A	yrd cdf	act Thr 205	tac	cat His	аса Тћг	sgt	500 GJu css	ggc ygu	сул аағ	yj9 aca	Jap qaA	195 791 345	cac His	tet Ser
9 <i>L</i> S	gct	tct	190 Ser fct	gj ddf	ATa Acq	ren	цуц зсд	tap qaA 281	зсс Трх	nəq ffd	дов Трг	ger	180 CJ Adf	atc Ile	стл аағ	aac Asn
828	crd	cat His 175	grt Val	дук зсд	occ Pro	gac Qac	110 CJ Adc	ysu ysu	дуг Туг	стл aaa	gct Ala	зсд Трг 39Т	gaa Glu	ваа Lys	gcg Ala	əya iii
085	jaa naA 160	nəq ççd	eyy adc	ràs sss	усс Трх	Jesc Yeb Ger	261 9dc	aca Thr	stc stc	aac Asn	120 A91 dfc	ааа Гуз	tss nsA	ст ^у аағ	aac Asn	gca Ala 245
432	gγλ ddc	eya Fre	2er fcd	tta Leu	140 F\x 999	gyn das	дре Трк	eyn dss	gtt Val	132 261 3df	дук Дук	ren cfd	ysb dsc	гуз	I30 ren	gyn ded

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	Trp	ь[А гí	nsA	Гел	εſΑ	Şex	neA 01	qıT	IJe	176	βīΑ	īγr S	IJe		I <0 nsA	
									S	rpţ:	ŗυατι	шеш	этів	9.8 T.5	3> A9 5> bi 7> 28 0> 18	<51: <51:
OLLT			590 290	Lgg Trp	C9d	tat Tyr	ejl aaf	gtc Val 585	ger Ser	gca Ala	ger Ser	дук Дук	280 GJÀ đđ¢	bye trc	cat His	суу Дар
1728		26r 26r 26r														
1680		att Ile														
1632		ejl ddc														
₹8 5 T	_	gjλ dd¢														
9891		gęd														
8871		sop slA 36‡														
7440		att 11e														
1392	GJ \\ ddc	dag gag	gac Asp	gat qeA	460 Val gtg	agc	tta Leu	дук Тук	ccc	gcg Ala 455	gat Asp	yya aca	стл aaa	gcg gcg	đ20 GJλ dđc	ctc Leu
1344	26r £cd	grr Val	ger ggc	tcc 26x	ъре ггг	сва	Pro ccd	зсс Тух	atg Met 440	ger Ser	асt Тhr	gcc PJ9	atc 11e	gac dsA 35	atc 11e	tss neA
1596	ggg	CJÀ đđc	asac Asn 05p	gac	дук ЗСС	att 11e	ejn dsd	425 11e 12e	yau yau	asc nsA	с _Ј у Дас	gcc gcc	aat Asn 420	att Ile	aac Asn	gfc Val
7548	дух Тух	988 619 615	gat qsA	atg JeM	gee Lys	суу даз	410 Lys	ger ggc	bro ccd	26r £cd	grt Val	aat Asn 30p	стл aac	ger ggc	atc Ile	gtc Val
	400 γ00	суу	zes	zes	сτλ	ьIA 29£	Λsl	εÍA	Γλε	zes	qsA 06£	геп	пеA	Trp	сту	385 385

Substitute Sheet (Rule 26) RO/AU

Thr Val Ala Thr Ala Val Leu Ala Thr Leu Leu Ser Ala Thr Val Cln

Val Val Val Ser Glu Leu Thr Arg Asn His Thr Lys Arg Ala Ser Ala $20\,$

380 375 Tyr Asp Val Asn Val Gly Asp Ala Leu Asn Val Asn Gln Leu Gln Asn 360 Thr Thr Ala Thr Val Ser Lys Asp Asp Gln Gly Asn Ile Thr Val Lys Clu Thr Val Thr Ser Gly Thr Lys Val Thr Phe Ala Ser Gly Asn Gly 330 Met Lys Thr Thr Ala Asn Gly Gin Thr Gly Gin Ala Asp Lys Phe Val Thr Ala Lys Glu Val Ile Asp Ala Val Asn Lys Ala Gly Trp Arg 562 rha cjh rha cjh cjn yau cjh ser ser tyr yab cjn cjh cjh ren Ala Lys Thr Ser Val Ile Lys Glu Lys Asp Gly Lys Leu Val Thr Gly yau Asi Giu Ser Lys Asp Asn Gly Lys Arg Thr Glu Val Lys Ile Gly Tyr Asp Thr Val Glu Phe Leu Ser Ala Asp Thr Lys Thr Thr Thr Val 230 Thr Gly Ser Thr Thr Gly Gln Ser Glu Asn Val Asp Phe Val Arg Thr SIZ Ser 11e Lys Asp Val Leu Asn Ala Gly Trp Asn 11e Lys Gly Val Lys 200 Ser His Val Asp Ala Gly Asn Gln Ser Thr His Tyr Thr Arg Ala Ala Yau Cjy ile Gly Ser Thr Leu Thr Asp Thr Leu Ala Gly Ser Ser Ala OLT Phe Ala Lys Glu Thr Ala Gly Thr Asn Gly Asp Pro Thr Val His Leu SST IPO Ala Asn Gly Asn Lys Val Asn Ile Thr Ser Asp Thr Lys Gly Leu Asn 132 Cin Leu Lys Asp Leu Thr Ser Val Glu Thr Glu Lys Leu Ser Phe Gly ren rha 11e rha Cju 2er Cjh rha Wap Phe Thr Tyr Ser Leu Lys Lys SOT His Asn Thr Leu His Gly Ala Thr Val Thr Leu Lys Ala Gly Asp Asn ein lie din ser Thr Gly Asp lie Gly Trp Ser lie Tyr Asp Asp 07. Arg Ser Ala Leu Val Leu Gln Phe Met Ile Asp Lys Glu Gly Asn Gly ςç Ala Asn Ala Thr Asp Thr Asp Glu Asp Glu Glu Leu Glu Ser Val Ala

Substitute Sheet (Rule 26) RO\AU

09 Ala Ser Ala Asn Asn Glu Glu Glu Glu Asp Leu Tyr Leu Asp Pro gca agt gct aac aat gaa gag caa gaa gaa tta tat tta gec ccc 192 07 Thr Val Lys Thr Ala Val Leu Ala Thr Leu Leu Phe Ala Thr Val Gln sec ded sad sec dec des ttg ges et etg ttg ttt ges aeg gtt eag 55T 52 Val Val Val Ser Glu Leu Thr Arg Asn His Thr Lys Arg Ala Ser Ala dre dre tee dad ete aea ege eae aea ege dee tee dea 96 0Τ Met Asn Lys 1le Tyr Arg 1le 1le Trp Asn Ser Ala Leu Asn Ala Trp std sac saa ata tac cgc atc att tgg aat gcc ctc aat gca tgg (222> (1)..(17)6) <551> CD2 <213> Neisseria meningitidis <212> DNA 9LLT <TTZ> <510> 50 282 Gly His Phe Gly Thr Ser Ala Ser Val Gly Tyr Gln Trp yab Iyr Giy Asn Irp Val Ile Lys Giy Thr Ala Ser Giy Asn Ser Arg Lyr Tyr Leu Gly Giu Ala Gly Tyr Ala ile Gly Tyr Ser Ser Ile Ser 015 232 yis gin his Ivr Leu Pro Gly Lys Ser Met Met Ala ile Gly Gly Gly 250 CJA yzu yja yxd yja CJA Ije yja CJU yja Ije yja Lpx yja CJA ren 909 ren rks cjk ket ein yau yau yau yau yau yau yau yau yau 061 Yau Asi Asi Pro Gly Val Lys Glu Gly Asp Val Thr Ash Val Ala Gln 0 L F Ala Leu Asn Val Gly Ser Lys Asp Ala Asn Lys Pro Val Arg Ile Thr ren cjh yja cjh yja ysb yja bro lyr len ser val ysp cju cjh 011 Yau 1je Yab 1je Yja Lyr Ser Wef Lyr Pro Gln Phe Ser Ser Val Ser 455 Val Asn ile Asn Ala Gly Asn Asn ile Glu ile Thr Arg Asn Gly Lys OTE Val 11e Ser Gly Asn Val Ser Pro Ser Lys Gly Lys Met Asp Glu Thr 368 390 Ser Gly Trp Asn Leu Asp Ser Lys Ala Val Ala Gly Ser Ser Gly Lys

Substitute Sheet (Rule 26) RO/AU

096	gct Ala 320	гуз гуз	aac Ash	gta Val	gca Ala	tsp qsA 21£	att Ile	∆gj ∂¢∂	ggs GJn	гуз Гуз	gca Ala 310	дук Тук	gtg Val	tta Leu	gjλ ddc	302 GJn 902
216	ej dac	gaa GJn	gac Asp	аса Тћг	300 Ser tct	tct	ejl aaf	tss nsA	egn dsd	595 Θ7λ άđc	ааа Гуѕ	gac qsA	ааа Гуѕ	gjλ dd¢	3 Трк Трк 3	grr Val
† 98	red red	rys sag	GJX ddr	gac Asp 285	ааа Lys	dsa GJ <i>n</i>	ааа Lys	att Ile	9tt Val 280	tot Ser	дрг Трг	aag Lys	gcg Ala	512 671 ddf	atc Ile	ggg
918	gtt Val	gaa GŢn	3сс Трг 072	aaa Lys	вая Гуѕ	gjλ ddc	aac Ash	gae Asp 262	aaa Lys	ger sdc	ggg	Λ ^g Ţ δ¢δ	tsa nsA 03S	gtt Val	дук Дук	Дух всд
89 <i>L</i>	aca Thr	999 Tys S25	рсе Тћг	dst AsA	gca Ala	Ser	Σ20 reπ rtd	ъре стс	egn død	gtc Val	дух Зся	gac Asp 245	rac Tyr	зсt Трк	grg	gtc gtc
720	S40 bye ffc	dst qsA	gtt Val	aac Asn	jsp qsA	Ser Ser	gct Ala	у Трх Трх	дух зсэ	сту Дағ	530 520 520	ggg Lys	gtt Val	ejl ddc	ggg Fys	316 116 225
Z <i>L</i> 9	aac Asn	dal tgg	GJ \\ ddc	gct	aac Asn 220	tta Leu	ara Agg	gac qsA	rys	215 Val 9tt	26r 9dc	gcs Ala	gcg &LA	cat	510 Fys	ggg Lys
ÞZ9	ejn ded	gac Asp	jap qaA	эсс Туг 205	grt Val	ysu ysu	gac gac	sac Asn	300 Трк ЗСС	gta Val	aac Ash	дрк Трк	асс Тhr	gcg Ala 291	ggy GJ	Дух чсс
919	1ss neA	ren cfd	190 ren crd	Дук чсд	jap qeA	дук зсс	nəq ftd	act Thr 285	261 fcd	gj ddf	att Ile	gŢλ dāŗ	sac Asn 180	cęd	cat His	grt Val
879	трк Трк	эсс Трт 275	dsc dsy	GJ Y ddc	aac Asn	дук чсд	710 CJ7 ddd	gct Ala	дса Трг	ggg	ааа Гуз	gcg Ala 261	ъуе ггг	taa neA	ttg Leu	ej ddc
081	333 Lys 160	дук ЗСС	yab dsc	261 9dc	аса Тhr	3fc 17 <i>6</i> 122	aac Asn	grc Val	aaa Lys	taa naA	120 CJ \ ddc	aac Asn	gca Ala	sgc	әча 111	142 Ser £cd
432	tta Leu	ggg	дзэ	зсг Трг	140 CJ V dds	gtt Val	agt Ser	дук ЗСС	ctg	tap qaA 281	дрк Трк	ctc	gac gac	ggg	ggg Lys	ren cçd
384	Ser	tac Tyr	зос Тух	152 bye ffc	asa naA	дук Зсв	gγλ ddc	aac Asn	150 Gju Css	ааа Гуѕ	atc 11e	saa Lys	ren crd	aac Asn 211	gac Asp	ejì dac
988	gcc gcc	ааа Гуѕ	110 ren	эсс Трх	atc Ile	dss ds	aga Arg	gcc Ala 201	аса Тћг	cta Leu	gta Val	ggy ddg	100 Lys	egn død	asc Asn	eya Frc
288	tat Tyr	gta Val 86	фју	Lrp tad	gat qaA	tca Ser	Jasi naA 06	два СТи	dsa G1n	gta Val	ggg	82 82 82	ggg	gyn dss	СŢλ dds	дук Дук
240	80 CTY ddc	дgg	ggg Lys	dst gsÅ	tcc	aat neA 37	Afc Afc	ata 911	ren ttd	Λ ^g Ţ ∂¢∂	gcc Ala 70	gtt gtt	дре Трг	yrd cdc	суу Суу	g£a Val 65

xliii

9441	tsa	£ãã	csd	tat	ddr	dfc	tot	дся	pot	dc£	đđε	בבכ	cst	aac	cđc	rcd
1728			tcc		_											
7680			tac Tyr													
7632			yys dcd													
₱8 S T	-		gca Ala	_			-	-							_	arg Arg
9887			970 176 210								-					gca Ala
1488			ург Трг													att 11e
T44O		-	_		_				-		_					₹62 СŢλ მმმ
1392		-					-		-	_	-					ger
DDEI																aac Asn
96ZT																dst gsA
1548																26r ccd
7500																382 ren cfd
1125																sct Thr
1104																GJY ddf
9501																osp gsA
1008																gγλ dd¢

Substitute Sheet UA/OA (82 eluA)

Val Thr Gly Lys Asp Lys Gly Glu Asn Gly Ser Ser Thr Asp Glu Gly 280 Lys lle Gly Ala Lyr Ser Val Ile Lys Glu Lys Asp Gly Lys Leu 597 Thr Thr Val Asn Val Glu Ser Lys Asp Asn Gly Lys Lys Thr Glu Val 220 Val Arg Thr Tyr Asp Thr Val Glu Phe Leu Ser Ala Asp Thr Lys Thr 235 230 Ile Lys Gly Val Lys Pro Gly Thr Thr Ala Ser Asp Asn Val Asp Phe 512 Lys Lys Arg Ala Ala Ser Val Lys Asp Val Leu Asn Ala Gly Trp Asn 200 Thr Gly Ala Thr Asn Val Thr Asn Asp Asn Val Thr Asp Asp Glu 38T Val His Leu Asn Gly Ile Gly Ser Thr Leu Thr Asp Thr Leu Leu Asn Gly Leu Asn Phe Ala Lys Glu Thr Ala Gly Thr Asn Gly Asp Thr Thr Ser Phe Ser Ala Asn Gly Asn Lys Val Asn Ile Thr Ser Asp Thr Lys 150 Gly Asp Asn Leu Lys Ile Lys Gln Asn Gly Thr Asn Phe Thr Tyr Ser bye wan Glu Lys Gly Val Leu Thr Ala Arg Glu Ile Thr Leu Lys Ala Thr Gly Glu Lys Glu Lys Val Glu Glu Asn Ser Asp Trp Ala Val Tyr Val Gln Arg Thr Val Ala Val Leu Ile Val Asn Ser Asp Lys Glu Gly Ala Ser Ala Asn Glu Glu Glu Glu Glu Asp Leu Tyr Leu Asp Pro 01 Thr Val Lys Thr Ala Val Leu Ala Thr Leu Leu Phe Ala Thr Val Gln 52 Val Val Val Ser Glu Leu Thr Arg Asn His Thr Lys Arg Ala Ser Ala 0.T Met Asn Lys lle Tyr Arg 11e 11e Trp Asn Ser Ala Leu Asn Ala Trp <213> Neisseria meningitidis <212> PRT

Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr Gln Trp 580 580

<211> 211> 21

Substitute Sheet (Rule 26) RO/AU

oligonucleotide primer for PCR <223> Description of Artificial Sequence: 5'

<213> Artificial Sequence

<212> DNA

<211> 21 <210> 22

	ДIL	290 GJu	TYr	сγλ	ΛgŢ	ger	61A 282	ser	ьlА	еуλ	әча	21H 082	суу	Arg	zez
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260 Ser	ser	τγr	gγλ	IŢG	ьIA ггг	ΤΫ́τ	сτλ	εſĄ	ејп	220 GJÀ	βıγ	Τλτ	πит	вуγ	242 GJÀ
етх	176	ьſА	тэМ	Met 540	zes	гλз	суу	ько	232 ren	ΤΥΥ	БÍĀ	ети	Λgλ	230 Fe <i>n</i>	сту
slA	дуд	ьſА	252 IJ6	БÍĀ	ети	БÍĀ	IJG	250 CJA	sſĄ	βzĄ	БĺĀ	nsA	272 CJ	qsA	ΛgŢ
nsA	qsA	210 IJ6	Arg	nsA	nsA	пеп	п еА 202	еји	БĹÁ	Val	ету	200 200	nəq	ети	БĺА
Val	nsA 261	дуц	Val	qsA	еул	4 60 CJn	гХз	Val	етх	ько	81 A 28≱	Val	nsA	дŲĮ	əĮI
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Substitute Sheet (Rule 26) RO\AU

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Substitute Sheet (Rule 26) RO\AU

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International application No.

INTERNATIONAL SEARCH REPORT

PCT/AU 98/01031

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	combination being obvious to a perso	tion or other means						
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	document of particular relevance; the be considered to involve an inventive	ch is cited to establish the publication date of "Y r citation or other special reason (as specified)						
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	-	continuation of Box C						
хэц	See patent family an	Further documents are listed in the						
ALL	\$627-582	VIRGI, M. et al. Mol Microbiol. 1992. 6(19): 27	¥					
ALL	A RUDEL, T. et al. Mature 1995. 373: 357-359							
TTV	A VIRGI, M. Adv. in Exp. Med and Biol. 1996. 408: 113-122							
Relevant to claim No.	propriate, of the relevant passages	Citation of document, with indication, where ap	*crogateD					
	J	DOCUMENTS CONSIDERED TO BE RELEVANT	c.					
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Form PCT/ISA/210 (second sheet) (July 1998) cophin

international application No.

INTERNATIONAL SEARCH REPORT

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mark on Protest The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.	ъъ
Mo required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Mos.:	'ቱ
payment of any additional fee. As only some of the required additional search fees were paid, specifically claims Nos.: report covers only those claims for which fees were paid, specifically claims Nos.:	.e
As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite	1.
is International Searching Authority found multiple inventions in this international application, as follows:	** *
	411
Z II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)	
Claims Mos.: Claims Mos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a) I I	
against themselves or their parent organism (Neisseria meningitidis). This concept is virtually meaningless. Claims Nos.: Decause they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)	Bo
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically: Claims 2, 3, 5, 6, 7, 9 are not clear. They are essentially to polypeptides which have immunological activity against themselves or their parent organism (Neisseria meningitidis). This concept is virtually meaningless. Claims Nos.: Claims Mos.: Claims Mos.: Claims Mos.: Claims Mos.:	3.
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically: Claims 2, 3, 5, 6, 7, 9 are not clear. They are essentially to polypeptides which have immunological activity against themselves or their parent organism (Neisseria meningitidis). This concept is virtually meaningless. Claims Nos.: Claims Mos.: Claims Mos.: Claims of their parent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)	.£
Claims Nos.: (A) 2, 3, 5, 6, 7, 9; (B) 20(1) and 21 Claims Nos.: (A) 2, 3, 5, 6, 7, 9; (B) 20(1) and 21 Claims Nos.: (A) 2, 3, 5, 6, 7, 9; (B) 20(1) and 21 Claims 2, 3, 5, 6, 7, 9 are not clear. They are essentially to polypeptides which have immunological activity against themselves or their parent organism (Neisseria meningitidis). This concept is virtually meaningless. Claims Nos.: Claims Nos.: Claims Nos.: Claims Nos.: Claims or their parent claims and are not drafted in accordance with the second and third sentences of Rule because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)	3. 3.
because they relate to subject matter not required to be searched by this Authority, namely: Claims Nos.: (A) 2, 3, 5, 6, 7, 9; (B) 20(1) and 21 because they relate to parts of the international search can be carried out, specifically: Claims 2, 3, 5, 6, 7, 9 are not clear. They are essentially to polypeptides which have immunological activity against themselves or their parent organism (Neisseria meningitidis). This concept is virtually meaningless. Claims 1, 2, 6, 7, 9 are not clear. They are essentially to polypeptides which have immunological activity against themselves or their parent organism (Neisseria meningitidis). This concept is virtually meaningless. Claims Nos.: Claims Nos.: Claims Nos.: Claims Nos.: Continued Conti	. 2. (A. 3. 3. 3. 3. 3. 3. 3. 3. 3. 3. 3. 3. 3.

Form PCTASA/210 (continuation of first sheet(1)) (July 1998) cophin

		i
hey lack support from the description	Claims 20(1) and 21 are to any antibodies against Neisseria meningitidis. T as they are not limited to antibodies to the polypeptides of the invention.	(B)
claims does not affect the search	Since these concepts are covered by other claims the lack of search on these coverage of the claims in toto.	
	(ii) antibodies to such antigenic polypeptides.	
aims 1, 4 or 7, which provide itidis infection, oτ	(i) antigenic polypeptides or their encoding nucleic acids according to cl protective immunity to an animal or human against Neisseria mening	
in from which they derive. However,	ens do not display immunological activity against themselves, or the organis as I can determine, these claims are intended to encompass either:	gitnA tst 28
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International applicati n No.	INTERNATIONAL SEARCH REPORT	

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